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MYCOLOGIA

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VOL. XLIX MAY–JUNE, 1957 No. 3

THE PERFECT STAGE OF AN ANTIBIOTIC-PRODUCING CEPHALOSPORIUM [1]

JAMES H. GROSKLAGS AND MARJORIE E. SWIFT

(WITH 9 FIGURES)

A laboratory contaminant isolated at the Michigan Department of Health in Lansing, and found to produce the antibiotic synnematin, was described in 1952 by Roberts (**5**) as *Cephalosporium salmosynnematum*. This culture (MDH *3590A* = NRRL *2271*) and two mutant derivatives of it were distributed to a number of institutions, including the University of Wisconsin and Lederle Laboratories, for further investigation. In 1955, in the course of cultural studies on a variety of agar media, the presence of ascocarps was noted independently at these two laboratories, not only in the original isolate but in the derived strains as well. Further studies have led to the classification of the fungus as a new species in the genus *Emericellopsis* van Beyma, family Aspergillaceae (Eurotiaceae).

Emericellopsis salmosynnemata sp. nov.

Cleistothecia globosa, glabra, 25–80(–120) μ diam.; paries cleistothecii subhyalinus, translucidus. Asci incomposite dispositi, globosi vel subglobosi, octospori, 13–18 μ, evanescentes. Ascosporae ellipsoideae, continuae, olivaceo-brunneae, (6–)7.5–8.5(–10) × 4–5(–5.5) μ, cum 3–5 alis vel cinctis linearibus 0.5–2 μ altitudine inter polos extantibus; membrana sporae plerumque aspera.

Status conidicus est *Cephalosporium salmosynnematum* Roberts (Mycologia **44**: 304. 1952).

[1] The portion of this study which was carried out at the University of Wisconsin was supported in part by a grant from Ely Lilly and Company.

[MYCOLOGIA for March–April, 1957 (**49**: 167–303) was issued April 30, 1957.]

Cleistothecia formed on cornmeal and oatmeal agar after 2–3 weeks at room temperature, abundant, visible macroscopically as very small black dots on the surface of the culture and within the medium, globose, glabrous, variable in size, 25–80(–120)μ diam., with a subhyaline, pseudoparenchymatous peridium through which the asci and spores may readily be viewed in position within the fruiting body. Asci few to many (only one or two in the smallest ascocarps), irregularly arranged, globose to subglobose, 13–18 μ diam., 8-spored, with an evanescent wall. Ascospores unicellular, olivaceous to brown, elliptical, 6–10 × 4–5.5 μ (most 7.5–8.5 × 4–5 μ), often with a large oil droplet, and provided with 3–5 longitudinal wings or flanges 0.5–2 μ wide, most of which extend from pole to pole of the spore and which have an undulating or sometimes ragged margin; the spore wall also often roughened with short papillae.

Homothallic; ascigerous stage obtained from single ascospores and single conidia.

The imperfect stage of this fungus is *Cephalosporium salmosynnematum* Roberts (Mycologia **44**: 304. 1952).

Type material has been deposited in the Herbarium of the University of Wisconsin, the Farlow Herbarium, and the collections at the Commonwealth Mycological Institute.

RELATED FORMS

The genus *Emericellopsis* was erected by van Beyma (**1**) in 1940, with *E. terricola* as the type species. Van Beyma (**1**) also described a variety, *E. terricola* var. *glabra*. Since that time several additional entities belonging to *Emericellopsis* have been discovered. *E. mirabilis* (Malan) Stolk was found in Italy and described in 1952 by Malan (**3**) as the type species of a new genus, *Peyronellula*. Stolk (**6**) later transferred it to van Beyma's genus and added another species, *E. minima*, from E. Africa. Recently Cain (**2**) erected the genus *Saturnomyces* for a fungus isolated from peat soil in Canada. The species *S. humicola* was described. However, Cain (private communication) now believes that this species should be transferred to *Emericellopsis*. One more entity is known. Orpurt (**4**) repeatedly isolated from Wisconsin soils an unknown member of the Aspergillaceae (Eurotiaceae) which he catalogued as "WSF *47*." Although Orpurt was unable to classify this fungus satisfactorily, it is now evident that it also is a member of the genus in question here and undoubtedly represents an additional species. All of the entities agree in having a *Cephalosporium* imperfect stage and in forming small globular ascocarps with a more or less transparent

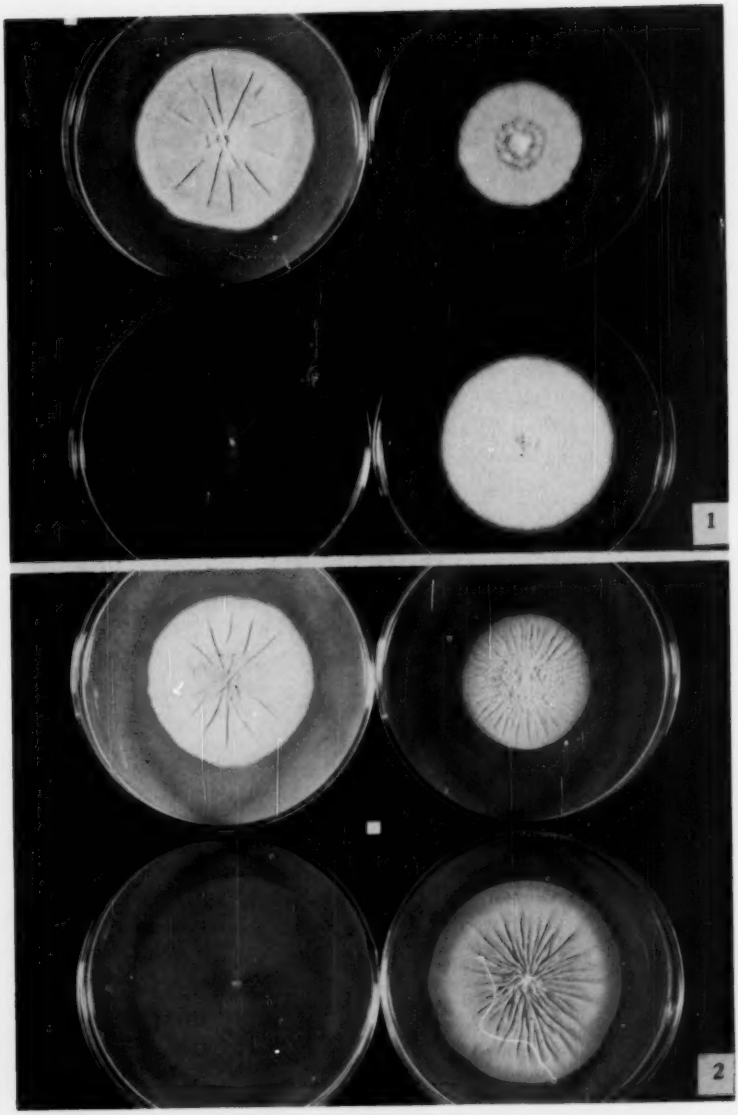
peridium (in some cases described as opening by a pore) and with scattered 8-spored asci. The ascospores are highly distinctive, being unicellular, elliptical, and dark-walled, with three to five prominent longitudinal wings.

All seven of the *Emericellopsis* entities enumerated above have been available to us for study in living condition. A careful comparison of them makes clear that this is a closely-related group of organisms, each of which nevertheless differs from all the others in certain important particulars, so that seven species appear to be distinguishable in the assemblage.

CULTURAL CHARACTERISTICS

Cultural characteristics of *Cephalosporium salmosynnematum* were recorded by Roberts (**5**). In the present investigation, this and other members of the *Emericellopsis* group were studied comparatively in plate culture on a variety of media, and some marked cultural similarities as well as some significant difference were noted among them. On Czapek-Dox (Cz), honey-peptone [2] (HP), and potato-dextrose (PD) agar, all except *E. humicola* and *E. mirabilis* show a pigmented growth ranging from pink or salmon to golden yellow. On PD and HP, however, colonies of WSF *47* eventually darken, particularly towards the center, as large numbers of ascocarps develop. On most of the media employed, with the notable exception of cornmeal (CM) and oatmeal (OM) agars, some radial folding of the mycelial mat is exhibited by a majority of the species, and such convolution of the colony is a pronounced feature in many cases. On Cz, *E. salmosynnemata* and *E. terricola* are almost indistinguishable (FIGS. 1, 2, 4), the resemblance extending to colony size, color, texture, and topography; but the two species are less alike on some other substrates. Under all cultural conditions explored, *E. minima* has been observed to grow appreciably faster than any of the other species, while *E. humicola* and *E. mirabilis* almost invariably have grown most slowly. Difference in the rate of radial extension of the mycelium is essentially the only character distinguishing one entity from another in young cornmeal agar cultures, where all species show a thin appressed growth that is hyaline or only faintly pigmented. In older CM cultures, however, development of the perfect stage in some cases provides cultural peculiarities which are diagnostic. Thus, WSF *47* can usually be picked out by a smoky color of the colony —a feature which is conferred by an exceptionally profuse development

[2] 6% honey, 1% Difco Bacto-peptone, 2% agar.



FIGS. 1, 2.

of small ascocarps, while larger fruiting bodies, often zonately arranged, characterize the cultures of *E. mirabilis* at this age.

CEPHALOSPORIUM STAGE

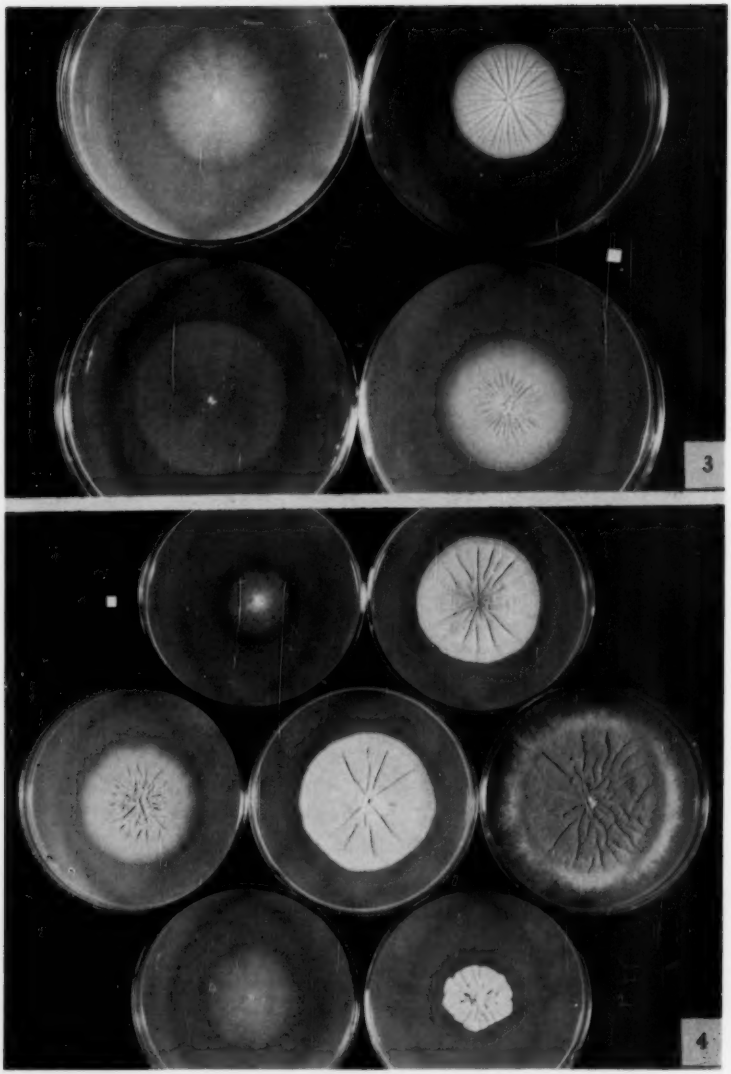
The writers cannot claim to have made an exhaustive study of the imperfect stage of the various *Emericellopsis* entities. However, it does not appear to them that the species could be distinguished with certainty on the basis of the asexual phase alone. As in most Cephalosporia, conidia are very variable in size and shape here, and in almost all of the previously described entities the authors have observed spores falling outside of the published dimensions. Furthermore, as has already been indicated by Roberts' (**5**) data, the size of conidia may be affected by the medium on which the fungus is grown. In the present study *E. minima* has sporulated very much more profusely than any of the other forms. It is by no means certain, however, that differences in quantity of asexual spores produced will prove to be a feature which can be used consistently to distinguish species in this genus. Great variation might, indeed, be expected in such a character if many isolates were studied, and it is a character which might readily be modified in an isolate maintained in culture over a long period of time. The original culture of *C. salmosynnematum* shows a strong tendency towards synnema production, as is suggested by the specific epithet applied by Roberts. But this same tendency is encountered in various other Cephalosporia and is not completely lacking in the other members of the *Emericellopsis* group. Nor is it by any means a constant feature in *C. salmosynnematum* itself. In cornmeal agar cultures, Roberts' original isolate almost invariably forms its conidia without synnema production, and some variant races obtained in our laboratories do not develop synnemata on any medium employed.

ASCOCARPIC STAGE

Although cultural characteristics and features of the conidial phase must naturally be given due consideration, it is in the perfect stage and particularly in the ascospores that are found the characters which appear to delimit most clearly the species of *Emericellopsis*.

Two percent cornmeal agar, prepared by adding agar to a filtered cornmeal decoction (40 gm yellow cornmeal in a liter of tap water

FIGS. 1, 2. 1. Upper row, left to right, *Emericellopsis salmosynnemata* on Czapek-Dox and honey-peptone agars; lower row, left to right, *E. salmosynnemata* on cornmeal and potato-dextrose agars. Cultures 2½ weeks old, incubated at 26° C. 2. Corresponding cultures of *E. terricola*.



FIGS. 3, 4. 3. Upper row, left to right, *Emericellopsis terricola* var. *glabra* on Czapek-Dox and honey-peptone agars; lower row, left to right, *E. terricola* var. *glabra* on cornmeal and potato-dextrose agars. Cultures 2½ weeks old, incubated at

heated over a water bath at 58° C for an hour), with or without a trace of yeast extract,[3] is an ideal medium for obtaining the perfect stage of *C. salmosynnematum;* oatmeal agar is only slightly inferior. These same substrates have also been found to support development of ascocarps in all six other *Emericellopsis* entities studied by the authors, although more fructifications of *E. terricola* were secured on an agar substrate prepared from baby's oatmeal cereal and tomato paste, while with *E. minima* neither these nor any of more than thirty other media tried were satisfactory in plate cultures. In the case of the latter species, ascocarps were finally obtained when the organism was grown on bottle slants of either cornmeal or oatmeal agar and the cultures were incubated for 6–8 weeks at alternating low and medium temperatures. The difficulty encountered in obtaining the perfect stage of *E. minima* was surprising in view of Stolk's (**6**) statement that this entity "readily produces perithecia on most media, especially oatmeal or cornmeal agar" and suggests that the isolate may have lost some of its original ability to fruit. The work carried out in these laboratories has shown that substrates such as Cz, HP, and PD agar, while supporting excellent vegetative growth, are generally unsuitable for production of the perfect stage in this genus. However, a notable exception is encountered in the case of WSF *47,* which fruits well on HP and profusely on PD. Some ascocarps of *E. salmosynnemata* have been obtained on PD, but only after an incubation period of several weeks. Limited fruiting of this species has also been secured on a modified Cz agar in which the salt concentration was reduced and in which cerelose was substituted for sucrose.

As has already been indicated, the ascocarps are basically very similar throughout the genus. The nearly black color which they show when a culture is viewed macroscopically or at low magnification is due almost entirely to the color of the spore mass within. Although van Beyma indicated that there is a pore in the ascocarp wall, this is either not consistently present or very inconspicuous, for the writers have been unable to detect a well-defined opening in the case of any species. The fragility of the ascus wall is indicated by the fact that intact asci are only rarely encountered when the contents of a mature fructification are crushed out in water on a slide. However, looking through the trans-

[3] The inclusion of yeast extract in very small amounts has the effect of reducing the time required for ascocarps to appear.

26° C. 4. Seven *Emericellopsis* entities on Czapek-Dox agar, 2½ weeks, 26° C. Center, *E. salmosynnemata.* Periphery, clockwise from white marker dot: *E. mirabilis, E. terricola, E. minima, E. humicola, E. terricola* var. *glabra,* and "WSF 47."

parent peridium of even a very old fruiting body, the spores are often observed in clumps of eight; and it may be presumed that each such cluster is still held within an ascal sac.

Except in a few special cases, ascocarp size is probably of limited value in defining taxa in this group. Fruiting bodies appear to vary greatly in magnitude in every species and, in some instances at least, their dimensions may be profoundly affected by the medium on which

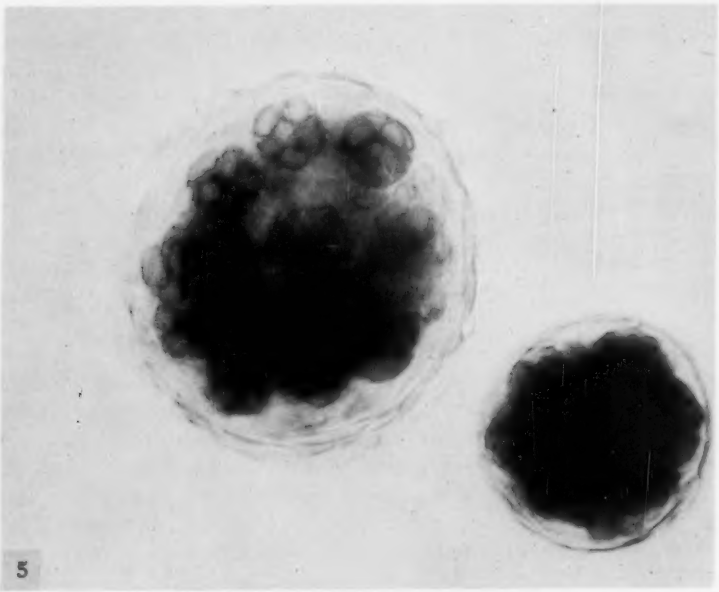
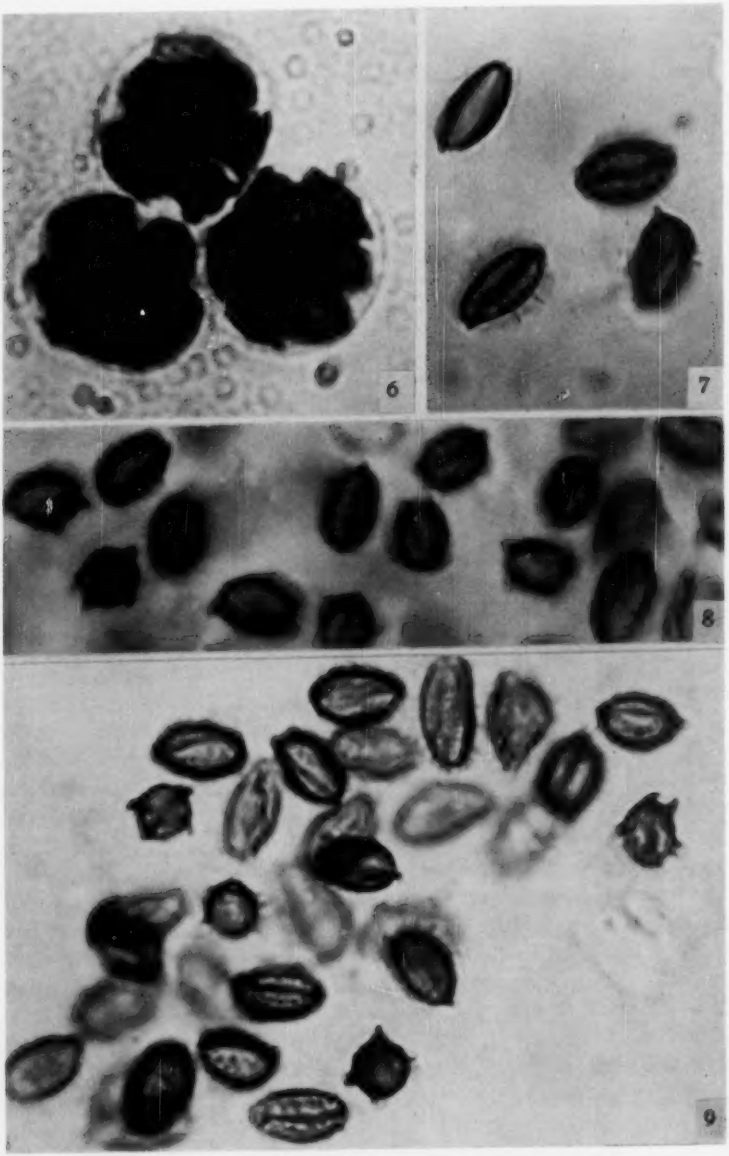


FIG. 5. Whole ascocarps of *Emericellopsis salmosynnemata*, × 750. Asci are visible through the transparent peridium.

the organism is growing. The largest ascocarps observed in the present study were some of *E. minima* measuring 200 μ in diameter, and the figures ("90–300 μ") given for this entity by Stolk (**6**) indicate ascocarps substantially larger than those produced by any other species. The fructifications of *E. salmosynnemata* with a diameter of 25–80(–120) μ, are in the general size range of those of *E. terricola*, *E. terricola* var. *glabra*, and *E. humicola*, according to the writers' observations. However, most of the ascocarps of *E. terricola* and *E. terricola* var. *glabra* obtained have been much smaller than the size suggested by van Beyma's

description ("80–90 μ"). In the case of both of these entities, as also with *E. humicola* and *E. salmosynnemata,* the majority of the cleistothecia in cornmeal agar cultures have measured less than 60 μ, with some as small as 25–30 μ. On the same substrate the fruiting bodies of *E. mirabilis* were found to be of considerably greater magnitude, many up to 130 μ and a few up to 185 μ having been recorded (although the dimensions given in the original publication on this species are "80–120 μ"). The pronounced influence of medium is illustrated in the case of WSF *47* where, on an average, cleistothecia produced in honey-peptone agar cultures have a diameter more than double that of the ones developed on cornmeal agar.

In last analysis, ascospore size and morphology seem to provide the most reliable basis for distinguishing the various *Emericellopsis* entities. *E. salmosynnemata* is readily distinguishable from *E. mirabilis, E. humicola, E. minima,* and WSF *47* solely on the basis of ascospore size, for the spores of *E. mirabilis* are very much larger and those of the three last named species very much smaller than the ones of the new taxon. The ascospores of *E. terricola* and *E. terricola* var. *glabra* also differ in size from those of *E. salmosynnemata,* both being somewhat larger; but the difference in this case is not sufficiently great so that the separation is easy on this character alone. However, the spore appendages and wall sculpturing provide supplementary features which aid in the distinction. The spores bear an equivalent number of appendages in each instance (3–5), but they appear to be more delicate structures in *E. terricola* than in the other two entities in question. Furthermore, the wings of both *E. terricola* and *E. terricola* var. *glabra* are wider than those of *E. salmosynnemata,* often extending out 2–3 μ and in end view of the spore appearing like "whips" attached to the wall, as van Beyma aptly expressed it; on the other hand, the flanges in *E. salmosynnemata* have not been observed to exceed 2 μ in width and most range nearer 1 μ, so that in end view they appear more horn-like than whip-like (FIGS. 8, 9). The typically strongly toothed margin of the wing in *E. terricola* also contrasts with the typically smooth undulating margin in the other two forms. However, in old spores, the "teeth" of *E. terricola* may become eroded and in *E. salmosynnemata* erosion of the wing margin may result in a somewhat ragged edge, with a consequent reduction in contrast in either case. The spore wing of *E. salmosynnemata* also often shows vein-like radial markings (FIG. 7) which are believed to be the result of folding or buckling of that membranous structure. Such markings have not been observed by the authors in the case of other species. One additional ascospore character remains to be mentioned; and while this, like



FIGS. 6–9.

the wing ribbing, is unfortunately a somewhat variable feature, it often makes possible quick recognition of the spores of *E. salmosynnemata*. Between the flanges, the spore wall in this species is frequently covered with short blunt emergences that appear to be more heavily pigmented than the rest of the wall (FIGS. 7–9). In contrast, the membrane is smooth or only inconspicuously roughened in *E. terricola* or *E. terricola* var. *glabra;* and, indeed, in none of the other known taxa of the genus is there to be found a comparable wall sculpturing.

ECOLOGICAL CONSIDERATIONS

The natural habitat of *Emericellopsis salmosynnemata* is unknown, the only isolate of this species having been obtained as a laboratory contaminant in Lansing, Michigan. All other known entities in the genus have been isolated from soil. Furthermore, most of the species appear to have been obtained from moist habitats—*viz., E. terricola* and *E. terricola* var. *glabra* from soil collected in Holland; *E. minima* from mangrove mud of the island of Inhaca, Mozambique, East Africa; *E. humicola* from peat soil at Thedford, Ontario, Canada; WSF *47* from low prairies in Wisconsin. *E. mirabilis* was isolated from vineyard soil in Italy. This information suggests where additional isolates of the known species—and perhaps some as yet undiscovered species—belonging to this genus might be sought. It is of interest that, while all of the other entities appear to have been isolated only once, Orpurt (**4**) isolated WSF *47* 109 times, in four different counties in Wisconsin, but always from wet prairies. It is probable that other members of the genus will also be found to be common soil inhabitants, once a search is made in the proper places.

ANTIBIOTIC PRODUCTION

Since the discovery of the perfect stage of *Cephalosporium salmosynnematum* made clear the true position of this organism among the fungi, and since the entire assemblage of forms comprising the genus *Emericellopsis* was seen to be a closely coherent group, which might therefore be expected to share some important physiological as well as morphological traits, the authors were prompted to test all the species for their ability to produce synnematin or some similar antibiotic. The tests, carried out on agar plates and in shaken flasks on suitable liquid media,

FIGS. 6–9. *Emericellopsis salmosynnemata*. 6. Asci, × 1400. 7. Ascospores, one showing wing folds (ribs?), × 1450. 8. Ascospores, × 1400. 9. Ascospores, × 1450, showing wings and papillate wall surface.

have revealed that a capacity to produce an antibiotic inhibitory to *Micrococcus pyogenes* var. *aureus, Salmonella typhimurium* and *S. gallinarum* is indeed widespread in the genus, positive results having been obtained with all of the seven known entities. Strong and consistent production of an antibiotic has been secured with WSF *47, E. terricola* var. *glabra,* and *E. salmosynnemata.* Somewhat weaker or sporadic activity has been recorded with the remaining four species.

SUMMARY

The perfect stage of *Cephalosporium salmosynnematum* Roberts, source of the antibiotic synnematin, has been obtained in cultures on cornmeal and oatmeal agar. On the basis of the character of this ascocarpic stage the fungus is described as a new member of the genus *Emericellopsis* van Beyma, *E. salmosynnemata,* in the family Aspergillaceae (Eurotiaceae). It is distinguished from other members of the genus especially by the dimensions and morphology of its ascospores. The fungus has been shown to be homothallic.

Six other taxa in the genus *Emericellopsis* were demonstrated to have some capacity to produce an antibiotic inhibitory to *Micrococcus pyogenes* var. *aureus, Salmonella typhimurium* and *S. gallinarum.*

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The writers wish to express their indebtedness to Professor Myron P. Backus for his generous assistance in the characterization of the new species. They also wish to thank Dr. C. R. Benjamin of the U. S. Dept. of Agriculture Northern Utilization Research Branch, Peoria, Illinois, and Professor Roy F. Cain of the University of Toronto for making available several of the cultures used in this study and for valuable taxonomic counsel.

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THE PARASITIC PHASE OF THE FUNGI OF CHROMOBLASTOMYCOSIS: DEVELOPMENT OF SCLEROTIC CELLS IN VITRO AND IN VIVO [1]

MARGARITA SILVA

(WITH 12 FIGURES)

Like many other fungi pathogenic to man, the fungi that cause chromoblastomycosis are dimorphic, *i.e.*, they have an extensive, filamentous, conidia-producing mycelium in the saprophytic phase, and in the parasitic phase, a much reduced thallus—small aggregates of spherical, brownish-yellow cells with thick, deeply-pigmented walls, dividing by septation rather than budding (FIGS. 1, 2). These structures, called sclerotic cells because of their thick walls, are believed to be highly resistant. Actual determinations of their vulnerability, however, are lacking and although their morphology is known in detail (**7**), the physiology of sclerotic cells is obscure, largely because this phase has not yet been elicited in artificial media, and is usually not obtainable in sufficient quantity from infected tissues.

In other dimorphic fungi, artificial induction of the parasitic phase has permitted some insight into the determinants of dimorphism (**3, 8, 9, 12, 15, 16, 18**). To understand the adaptation of a fungus to parasitism it is necessary to identify the factors governing the appearance of the parasitic type of morphology. Success here might aid in the development of a rational therapy for the disease.

Methods were sought, therefore, to induce the parasitic phase regularly and in quantity. The studies were conducted both *in vitro* and *in vivo*.

MATERIALS AND METHODS

A basic selection of nineteen isolates, representing the most commonly found agents of chromoblastomycosis, was employed (TABLE I).

For the *in vitro* work, two things were sought: (1) a culture medium that would imitate the habitat of these fungi in human cutaneous tissue

[1] Portion of a thesis accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Radcliffe College (Harvard University)

fluids, and (2) to test the applicability to the fungi of chromoblastomycosis of media previously shown to elicit the "parasitic" morphology of other dimorphic fungi.

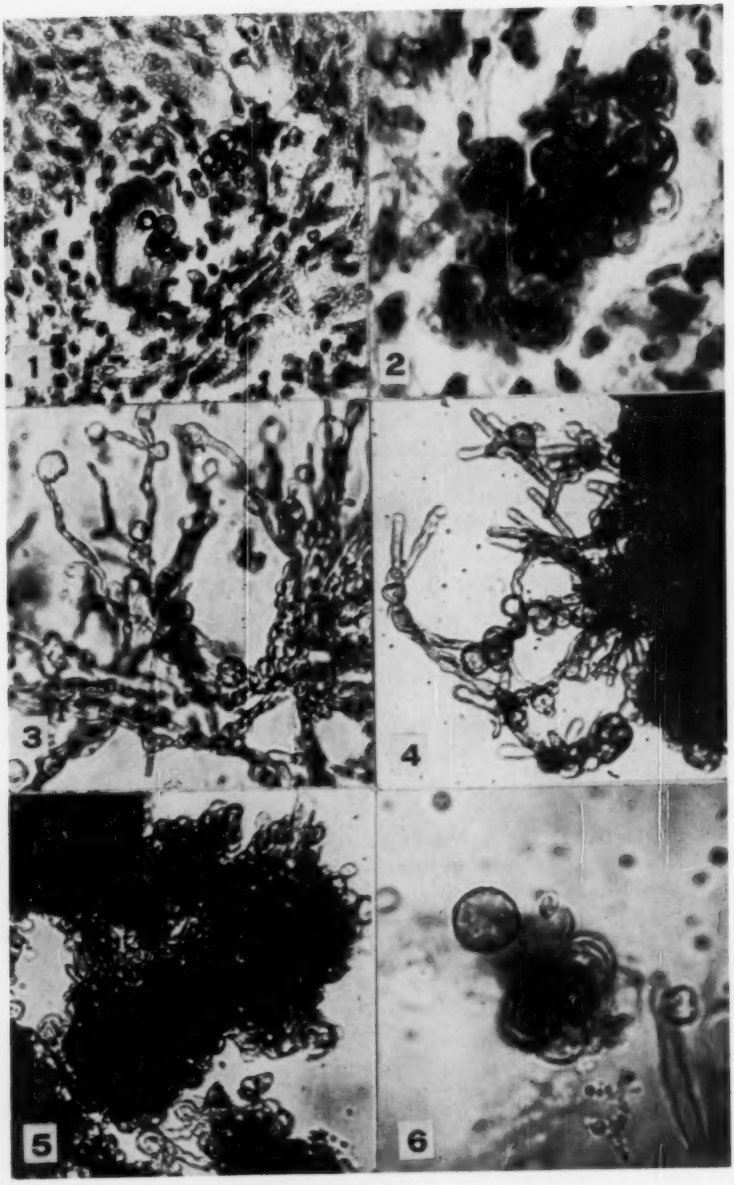
For the first objective, a "synthetic lymph" was prepared having the following composition: Distilled water, 990 ml; glucose, 0.70 g; urea, 0.18 g; NaCl, 7.0 g; $MgCO_3$, 0.10 g; $CuSO_4 \cdot 7H_2O$, 0.035 g; K_2HPO_4, 0.38 g; $Ca(NO_3)_2 \cdot 4H_2O$, 0.31 g; K_3PO_4, 0.072 g; $Ca_3(PO_4)_2$, 0.03 g. The medium was adjusted to pH 7.2 with HCl and sterilized by autoclaving. The protein component of cutaneous tissue fluids was supplied as blood plasma. Keratin, a component of skin, was added as a few bits of sunburn peelings or toe-nail clippings; these materials were sterilized by immersion in 70 percent alcohol for 30 minutes and dried with sterile paper towels before addition to media. Other supplements were added aseptically to the liquid basal medium; in some experiments the viscosity of the media was increased by means of agar.

The following supplements were used:

10 percent human blood plasma
10 percent plasma plus 0.18 percent agar
50 percent plasma
50 percent plasma plus 0.10 percent agar
10 percent plasma plus 0.18 percent agar, plus bits of sunburn peelings
10 percent plasma plus 0.18 percent agar plus bits of toe nail.

For the second objective two media were tested. One was the triple peptone medium used by Salvin (**17**) for inducing the yeast-like, parasitic type of growth in *Histoplasma capsulatum*. The second medium was Francis' glucose-cystine blood agar; with it Campbell had elicited conversion from the hyphal to the yeast-like form in *Sporotrichum Schenkii* (**5**) and *H. capsulatum* (**6**).

Media were dispensed in test-tubes or flasks. With the two semi-fluid media, 10 ml amounts were employed in 25 × 100 mm test tubes, or 25 ml amounts in 100 ml Erlenmeyer flasks, thus forming shallow layers. Incubation for 3 weeks was either at room temperature or at 37° C. The culture vessels were either kept stationary or agitated continuously in a reciprocal shaker. The Francis agar was dispensed in 150 × 20 mm tubes, slanted to present a large surface; such cultures were incubated at 37° C. For inocula, mycelium and spores of the saprophytic forms of these fungi were scraped from the surfaces of 3-week-old colonies on Sabouraud's glucose agar. As indicated, inocula



FIGS. 1–6.

TABLE I

CLASSIFICATION AND ORIGIN OF ISOLATES *

A. *Fonsecaea Pedrosoi* (Brumpt) Negroni *emend.* Carrión.

1. *Fonsecaea Pedrosoi* var. *Pedrosoi.*

S-399C Brazilian isolate, "*Hormodendroides Pedrosoi*" of Moore and Almeida, 1936.
S-399D Brazilian isolate, "*Botrytoides monophora*" of Moore and Almeida, 1936.
S-399F Argentinian isolate, Negroni, 1936.
S-399N South African isolate, Simson *et al.*, 1943.

2. *Fonsecaea Pedrosoi* var. *Cladosporioides* Carrión.

S-376 Algerian isolate, "*Hormodendrum algeriensis*" of Montpellier and Catanei, 1927.

3. *Fonsecaea Pedrosoi* var. *Phialophorica* Carrión.

S-954 Brazilian isolate, "*Phialophora macrospora*" of Moore and Almeida, 1936.

4. *Fonsecaea Pedrosoi* var. *communis* Carrión.

S-399E Isolate obtained from the Centraalbureau voor Schimmelcultures at Baarn, Holland, in 1939, labelled as "*Trichosporium Pedrosoi*," probably of Brazilian origin.
S-399H Puerto Rican isolate, 1940.
S-399Q Puerto Rican isolate, 1941.
S-399R and S-399T Puerto Rican isolates, 1943.
S-399V Puerto Rican isolate, 1945.

B. *Fonsecaea compacta* (Carrión) Carrión.

S-368 Tennessee Strain, isolated in 1944 by Lt. Col. Ferraro, at Camp Forest, Tenn., obtained from Dr. N. F. Conant.
S-369 Puerto Rican isolate, 1934.

C. *Phialophora verrucosa* Medlar.

S-950 Texas isolate, Wilson *et al.*, 1933.
S-951 Boston isolate, Medlar and Lane, 1915.
S-952 Uruguayan isolate, MacKinnon, 1934.
S-955 Isolate received from Dr. Morris Moore, St. Louis, Mo., in 1939.
S-957 Isolate received from Dr. F. D. Weidman, Philadelphia, Pa., in 1940.

* The nomenclature and taxonomic treatment in this TABLE follows that proposed by Carrión in 1940 (Puerto Rico Jour. Pub. Health Trop. Med. **15**: 340–361) and 1942 (Mycologia **34**: 424–441).

were sometimes obtained by serial transfer from cultures partially converted to the parasitic phase.

For the *in vivo* work, the search for an adequate supply of sclerotic cells representative of the parasitic phase was extended to a search for an experimental animal that might yield such a supply.

FIGS. 1–6. 1, 2. Sclerotic cells of *Fonsecaea Pedrosoi* in human skin, × 430 and × 970, respectively. 3–6. Progressive development of the parasitic, sclerotic-cell type of thallus from the filamentous form in *Phialophora verrucosa* (S-955) on Francis' glucose-cystine blood agar at 37°C; 3, 4 and 5, × 430; 6, × 1200.

TABLE II

DEVELOPMENT OF SCLEROTIC CELLS ON "SYNTHETIC LYMPH" BY FOUR ISOLATES

Additions to basal medium	Conditions of incubation	Isolates			
		Fonsecaea compacta S-368	*F. Pedrosoi* var. *communis* S-399E	*F. Pedrosoi* var. *Pedrosoi* S-399F	*F. Pedrosoi* var. *communis* S-399H
*a**	37° C., Stationary	20% S.C.**	25% S.C.	0% S.C.	5% S.C.
a	Room T., Stationary	15% S.C.	0% S.C.	15% S.C.	1% S.C.
b	37° C., Stationary	1% S.C.	25% S.C.	5% S.C.	0% S.C.
b	Room T., Stationary	0% S.C.	10% S.C.	1% S.C.	0% S.C.
b	Room T., Agitated	—	0% S.C.	0% S.C.	—
c	37° C., Stationary	—	0% S.C.	no growth	—
d	37° C., Stationary	—	0% S.C.	5% S.C.	—
e	Room T., Agitated	—	0% S.C.	0% S.C.	—
e	Room T., Stationary	—	10% S.C.	1% S.C.	—
e	37° C., Stationary	—	10% S.C.	0% S.C.	—
f	37° C., Stationary	—	10% S.C.	5% S.C.	—
f	Room T., Stationary	—	10% S.C.	0% S.C.	—

* Key to additions: *a* = 10% plasma; *b* = 10% plasma + 0.18% agar; *c* = 50% plasma; *d* = 50% plasma + 0.10% agar; *e* = 10% plasma + keratin; *f* = 10% plasma + 0.18% agar + toenail bits.

** S.C. = sclerotic cells, approximate percentage determined by microscopic examination of 3-week-old cultures.

TABLE III

DEVELOPMENT OF SCLEROTIC CELLS BY 19 ISOLATES ON TRIPLE PEPTONE MEDIUM

Classification	Isolate	Percent sclerotic cells and conditions of incubation	
		37° C., stationary	Room temperature agitated
Fonsecaea Pedrosoi var. *communis*	S-399E	1*	5
Fonsecaea Pedrosoi var. *communis*	S-399H	10	10
Fonsecaea Pedrosoi var. *communis*	S-399Q	0	—
Fonsecaea Pedrosoi var. *communis*	S-399R	0	—
Fonsecaea Pedrosoi var. *communis*	S-399T	0	—
Fonsecaea Pedrosoi var. *communis*	S-399V	5	—
F. Pedrosoi var. *Cladosporioides*	S-376	0	—
F. Pedrosoi var. *Pedrosoi*	S-399C	0	—
F. Pedrosoi var. *Pedrosoi*	S-399D	0	—
F. Pedrosoi var. *Pedrosoi*	S-399F	5	no growth
F. Pedrosoi var. *Pedrosoi*	S-399N	0	—
F. Pedrosoi var. *Phialophorica*	S-954	0	—
F. compacta	S-368	50	90
F. compacta	S-369	5	—
Phialophora verrucosa	S-950	5	—
Phialophora verrucosa	S-951	0	—
Phialophora verrucosa	S-952	0	—
Phialophora verrucosa	S-955	25	—
Phialophora verrucosa	S-957	1	—

* Approximate percentage sclerotic cells determined by microscopic examination of the growth after 3 weeks' incubation.

The same group of 19 isolates used for the studies *in vitro* (see TABLE I) was employed, and in addition, certain other related pathogenic and two saprophytic species of fungi were used, where indicated, for comparison. The animals tested for the suitability of their tissues as a substrate for the growth of sclerotic cells included the embryonated hen's egg, the pupae of two species of silkworm, and the albino mouse.

The methods of inoculation will be discussed below for each species of animal.

TABLE IV

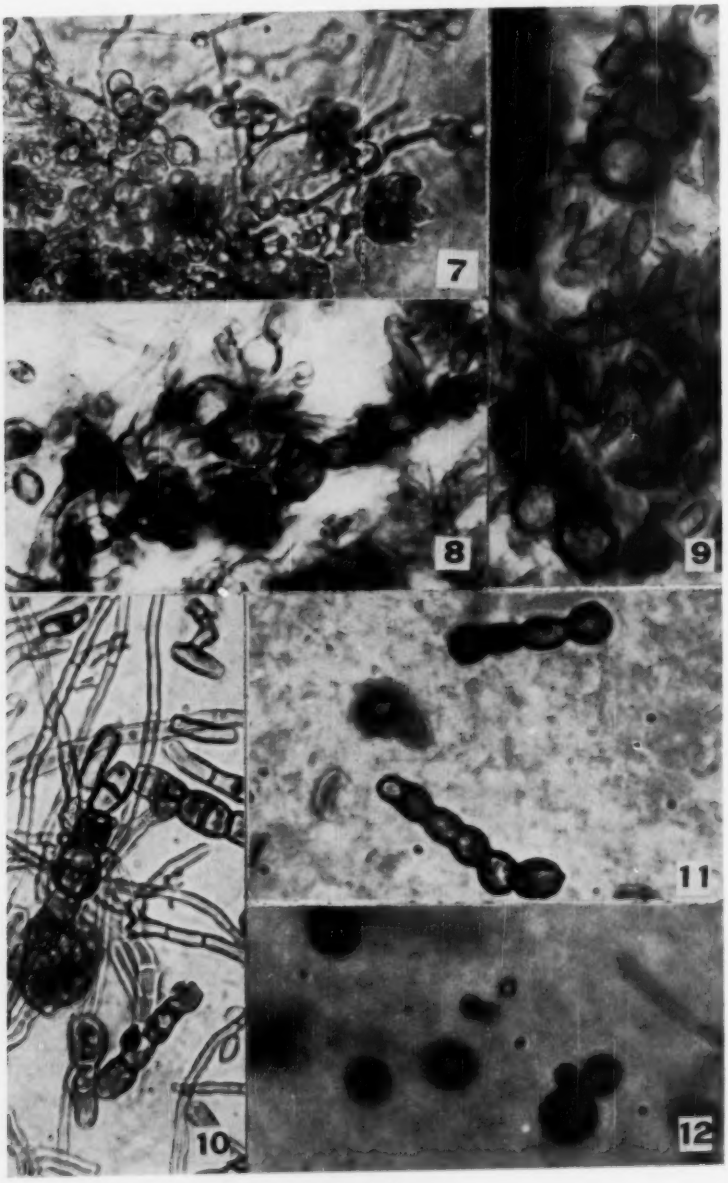
DEVELOPMENT OF TWO ISOLATES ON FRANCIS' GLUCOSE-CYSTINE BLOOD AGAR AT 37° C

Transfer number	Age	Isolates	
		S-399E	S-955
		Fonsecaea Pedrosoi var. *communis*	*Phialophora verrucosa*
T-1	1 wk.	Colonies velvety, filamentous	Few papillate colonies and sclerotic cells
	2 wks.	Colonies velvety, filamentous	Additional papillate colonies and s. c.*
	3 wks.	Colonies velvety, filamentous	Further increase in number of papillate colonies and s. c.
T-2	1 wk.	Few papillate colonies and sclerotic cells	Papillate colonies and s. c. become more numerous than on first transfer and increase with age.
	2 wks.	Few papillate colonies and sclerotic cells	
	3 wks.	Few papillate colonies and sclerotic cells	
T-3	1 wk. to 3 wks.	No increase in number of papillate colonies or of s.c. either with subculture or aging	Progressive increase in the number of papillate colonies to an almost total disappearance of the velvety type of growth. Masses of spherical cells gradually replace the filamentous mycelium
T-4	1 wk. to 3 wks.	No increase in number of papillate colonies or of s. c. either with subcultures or aging	Progressive increase in the number of papillate colonies to an almost total disappearance of the velvety type of growth. Masses of spherical cells gradually replace the filamentous mycelium
T-5	1 wk. to 3 wks.	No increase in number of papillate colonies or of s. c. either with subcultures or aging	Progressive increase in the number of papillate colonies to an almost total disappearance of the velvety type of growth. Masses of spherical cells gradually replace the filamentous mycelium

* s. c. = sclerotic cells.

RESULTS

1. *Development in "synthetic lymph."* The mineral-base blood plasma medium used as a "synthetic lymph" was tested with four isolates. The growth obtained was primarily filamentous in most instances; in some cultures spherical intercalary chlamydospores were unusually abundant. These chlamydospores had internal septa in more than one plane, thick walls, and were more deeply pigmented than neighboring elongated hyphal elements; indeed, they resembled the sclerotic cells formed in the host. The ratios of these spherical chlamydospores (*i.e.* sclerotic cells) to ordinary cylindrical hyphal cells formed by the



FIGS. 7–12.

four isolates on the various modifications of this medium are shown in TABLE II. Sclerotic cells did not appear consistently.

2. *Development in the triple peptone medium of Salvin.* In this medium, which was tested with the 19 isolates, again thick-walled chlamydospores developed. TABLE III lists the proportions of such chlamydospores for each isolate. This medium was also unsatisfactory for adequate conversion to the parasitic type of morphology.

3. *Development in Francis' glucose-cystine blood agar.* This medium was the most promising of all: it induced the most nearly complete conversion to the parasitic phase so far seen here. Two isolates were chosen for study on this medium because of their previously observed greater tendency to form sclerotic cells from the filamentous mycelium; an isolate of *Fonsecaea Pedrosoi* var. *communis* (S-399E), and an isolate of *Phialophora verrucosa* (S-955). TABLE IV summarizes their development during successive transfers on the glucose-cystine blood agar; while only a slight conversion was obtained with isolate No. S-399E, the parasitic type of morphology was elicited quite successfully in isolate No. S-955 (FIGS. 3–6).

4. *Development in the embryonated hen's egg.* The chick embryo's chorioallantoic membrane, a layer of epithelial cells overlying a highly vascularized mesodermal layer, seemed an ideal tissue to elicit the parasitic phase, since this tissue resembles the human dermis and epidermis where these fungi regularly form the reduced, sclerotic-cell type of growth. In addition, the success reported by Moore (**14**) in obtaining the conversion of one strain of *Phialophora verrucosa* to its parasitic morphology when subcultured on the chick chorioallantois, further suggested testing this tissue as a substrate for obtaining large numbers of sclerotic cells.

The technique employed followed essentially that described by Goodpasture and Buddingh (**10**) and by Burnet (**4**) for the study of viruses, and later applied by Moore (**13**) to the study of human pathogenic fungi. Inocula of various ages, obtained from either slant cultures on Sabouraud's glucose agar, or from previously infected membranes, were intro-

FIGS. 7–12. Development of sclerotic cells by the fungi of chromoblastomycosis in experimentally-infected animal tissues. 7. Multiple spherical chlamydospores (sclerotic cells), some showing internal septation, developed on the mycelium of *Phialophora verrucosa* (S-955) in chick chorioallantois, × 600. 8, 9. Spherical chlamydospores (sclerotic cells), some with eosinophilic deposit on outside of wall, on the mycelium of *Fonsecaea Pedrosoi* (S-399E) in silkworm pupae, × 1200. 10–12. Progressive development of sclerotic cells from the filamentous mycelium of *Fonsecaea Pedrosoi* (C-31) in liver of albino mouse, × 970. (Photomicrographs in FIGS. 10 and 11 taken with a green filter.)

duced by means of a capillary pipette through a small window cut on the shell directly over the collapsed chorioallantoic membrane.

Inoculation was made at various stages between the 10th and 16th day of embryonic development and the membranes harvested 5 to 11 days after inoculation in a search for the most susceptible time for infection as well as the time required for conversion of the fungus to the parasitic phase. In addition to the 19 basic fungous isolates, two saprophytic strains of *Cladosporium* (Nos. H-48-2 and H-48-8, air contaminants) were used because of their morphologic resemblance to those fungi of chromoblastomycosis which exhibit the *Hormodendrum-Cladosporium* type of sporulation (**7**), and also two isolates of *Torula Bergeri* (S-1074 and S-1077), from a Canadian case of the disease

TABLE V

DEGREE OF CONVERSION OF ELEVEN ISOLATES TO THE PARASITIC PHASE IN THE CHORIO-ALLANTOIC MEMBRANES OF THE CHICK EMBRYO

Isolate	Conversion obtained in	Amount of conversion (Percentage of sclerotic cells)
Fonsecaea compacta		
S-368	4 out of 11 membranes	5 and 10% sclerotic cells
F. Pedrosoi var. *communis*		
S-399E	5 out of 10 membranes	2, 10 and 75% sclerotic c.
S-399R	1 out of 4 membranes	1–2% sclerotic c.
S-399P	2 out of 5 membranes	1 and 10% sclerotic c.
S-399V	1 out of 7 membranes	1% sclerotic c.
$S\text{-}399V_1$	1 out of 6 membranes	1% sclerotic c.
Phialophora verrucosa		
S-950	1 out of 8 membranes	1% sclerotic c.
S-952	3 out of 5 membranes	10 to 15% sclerotic c.
S-955	17 out of 62 membranes	1, 5, 10, 18, 25, 30, 60 and 85% sclerotic cells
*Torula Bergeri**		
S-1077	2 out of 4 membranes	100% sclerotic cells*
Hormodendrum sp. (saprophytic)		
H-48-8	1 out of 1 membrane	1% sclerotic cells

* This fungous isolate is not filamentous *in vitro* either.

(**1, 2**), because they represent the yeast-like type of isolate from chromoblastomycosis. Finally, one isolate each of *Pleurage curvula* (H-48-0) and *Pleurage zygospora* (H-48-11) were investigated since the spermatiophores produced by them had been observed to be identical in morphology to the *Phialophora* type of conidiophore produced by the saprophytic phase of some of the fungi of chromoblastomycosis.[2]

The fungal growth and the response of the membranes to the presence of the pathogen were variable. In some instances, no infectious response was induced and the fungus developed morphologically as a saprophyte, producing a rapidly growing colony with a dark gray-to-black filamentous mycelium. Where infection occurred, the chorioallan-

[2] C. M. Wilson. Personal communication, 1949.

toic membranes showed a marked inflammation; they became thickened and stiff, lost their hyaline appearance, and showed increased vascularization and a hemorrhagic reaction. Lesions appeared in the form of discrete grayish spots, somewhat embedded in the tissues of the host and containing variable amounts of the fungus.

Microscopically the pathogen appeared as a network of branching hyphae with intercalary or terminal, thick-walled, spherical and septate enlargements (*i.e.*, chlamydospores) resembling the sclerotic cells observed in human tissue (FIG. 7).

The degree of conversion from the filamentous to the sclerotic cell phase was estimated by counting 100 fungal elements in an NaOH mount of a portion of infected membrane. Each fungous element was arbitrarily classified as either "sclerotic cell" or "hyphal element," the latter if it was of normal diameter and cylindrical in shape, the former if it was spherical and enlarged. These readings are summarized in TABLE V. Sclerotic cells were seen in 11 of the 25 isolates and comprised from 1 to 85 percent of the fungal elements. The amount of sclerotic cells from a single isolate varied from embryo to embryo: there might be many, few or none. The most promising isolate was a strain of *Phialophora verrucosa,* No. S-955, received from Dr. Morris Moore, and probably the same culture yielding sclerotic cells in similar studies (**14**). In the present study, 62 chorioallantoic membranes were inoculated with this isolate, either directly from agar cultures or by serial transfer from previously infected membranes. Sclerotic cells appeared in only 17 of the 62 membranes, and although conversion with this isolate reached 85 sclerotic cells per 100 fungous elements, this high yield was only obtained once, and was not duplicated in subsequent inoculations, even though the conditions of the experiment that produced them were imitated as closely as possible.

Inability to reproduce high yields of sclerotic cells was also true of other isolates, and although attempts were made to find conditions for consistent conversion to the parasitic morphology, variations in age, size or source of inocula, age of embryo at time of inoculation, or number of days elapsing before the membranes were harvested, all failed to solve the problem.

Nevertheless, some correlation did appear between the origin of the isolates and the ability to grow at all in the membranes. Of the isolates that grew, only two did not belong to the original group of 19 isolates from chromoblastomycosis. These were No. S-1077 (*Torula Bergeri*) and No. H-48-8 (saprophytic *Cladosporium*). Neither of the two isolates of *Pleurage* (*P. zygospora* and *P. curvula*) grew in the tissues of

embryonated eggs nor injured the developing embryos. This isolate of *P. curvula* was also tested by Sussman (**19**) for its infectivity to the pupae of the silkworm, *Platysamia cecropia;* there, too, it did not proliferate.

An abundant supply of the parasitic phase of these fungi might perhaps be attained if fresh isolates that have not become "adapted" to a saprophytic way of life on artificial media were employed. The ideal procedure would be to transfer infected material directly from the human host and attempt to transmit the infection as such to the chick chorioallantoic membrane.[8]

5. *Development in the pupae of two species of silkworm.* That pupae of the silkworm *Platysamia cecropia* can serve as "living test tubes" for cultivation of pathogenic fungi was demonstrated by Sussman (**19**). In testing an array of pathogens, Sussman included two of the writer's chromoblastomycosis isolates, Nos. S-399E and S-399R of *Fonsecaea Pedrosoi* var. *communis.* Pupae infected with these two isolates were examined in the present study and were found to permit the growth and proliferation of both fungi; the growth sometimes proved fatal. Upon microscopic examination of the infected tissue it was found that both pathogens had undergone partial conversion to the parasitic phase, as indicated by an abundance of heavy-walled sclerotic cells (FIGS. 8, 9), some of which had a crusty, eosinophilic deposit on their surface (FIG. 9) similar to that sometimes observed in sclerotic cells infecting human tissues (**20**).

These findings suggested further trial of this insect as a source of sclerotic cells. Twelve pupae of *Platysamia cecropia,* secured by Dr. Sussman through the generosity of Prof. C. M. Williams of Harvard University, were inoculated with the following isolates: *Fonsecaea Pedrosoi* var. *communis* (S-399E, S-399R and S-399V), *F. compactum* (S-368) and *Phialophora verrucosa* (S-957). All these isolates grew in the insect tissue, forming a varying number of sclerotic cells; complete conversion to the parasitic phase was never obtained.

Similar studies were made with pupae of the related species of silkworm, *Philosamia cynthia.* Two isolates of *Torula Bergeri,* the yeast-like organisms obtained from a Canadian case of chromoblastomycosis (**1, 2**), were employed. These two organisms also were able to grow inside the pupae, producing mycelium and yeast-like cells. The infected

[8] Since this manuscript was written, the successful cultivation of the fungi of chromoblastomycosis in their parasitic phase in the yolk-sac of the chick embryo has been reported by Howles *et al.* (**11**), who employed as inoculum sclerotic cells contained in the skin and exudates from human cases of the disease.

pupae continued to live. Some of them underwent metamorphosis into adult moths; evidently these two isolates were of very low virulence. The adult moths looked healthy and were not examined internally for infection.

6. *Development of a freshly-isolated strain of Fonsecaea Pedrosoi in the albino mouse.* To test the conversion to the parasitic type of morphology in laboratory animals of a newly-isolated strain of *Fonsecaea Pedrosoi,* C-21 in our collection, 4 young albino mice were inoculated intraperitoneally with suspensions of aerial mycelium and spores from a primary culture of this fungus (freshly isolated from a patient) on Sabouraud's glucose agar. The mice were sacrificed and autopsied at the end of 1, 2, 4, and 8 weeks after inoculation. Systemic lesions were found—small abscesses on the spleen, intestinal mesentery and liver. These lesions did not develop rapidly, nor were they persistent, for the most conspicuous lesions were found in the mouse autopsied after 4 weeks. In this animal, the fungous elements were converted partially from the filamentous to the sclerotic cell form. The exudate from a lesion in the liver, in a NaOH preparation, had areas where the conversion of filaments to sclerotic cells could be observed (FIGS. 10–12). The first change observed in such filaments was thickening of the walls and an increase in the number and frequency of the septa (FIG. 10); this was followed by a rounding up of the hyphal articles to form chains of spherical elements (FIG. 11) and finally, by fragmentation of these chains to form individual sclerotic cells (FIG. 12).

DISCUSSION

Sclerotic cells resembling those produced by the fungi of chromoblastomycosis in the human skin were obtained *in vitro* and *in vivo* in variable numbers depending on the isolate and on the substrate employed for its cultivation. While large quantities of sclerotic cells were not obtained consistently, our observations furnish some insight as to how the sclerotic cells originate from the filamentous form—a point of fundamental interest. In preparations from cultures, the thick-walled spherical structures were seen to originate as chlamydospores by the rounding out of an intercalary or terminal element of a vegetative hypha (FIG. 3), and sometimes by the rounding out of a conidium, with notable thickening of the wall. The formation of adjacent rounded elements (FIG. 4), the appearance of internal septa (FIG. 5), and the final separation of a cluster of spherical cells (FIG. 6), further increased the analogy between these structures and the sclerotic cells found in tissue —the evidence clearly indicating that the parasitic phase of these fungi

in vivo may originate in a similar manner through the formation of chlamydospores from the inoculated vegetative mycelium or conidia of the saprophytic phase. The results on experimental animals further confirmed this interpretation.

Although the mode of conversion of the vegetative mycelium to sclerotic cells was clearly seen from these experiments, the factors required for the induction of the parasitic phase of the fungi of chromoblastomycosis must still be determined.

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ROUTINE IDENTIFICATION OF CANDIDA ALBICANS: CURRENT METHODS AND A NEW MEDIUM [1]

CLAIRE L. TASCHDJIAN

Proper identification of a yeast-like organism isolated from the mucous membranes, the feces, or the skin in the absence of clinical manifestations is of practical importance. Failure to differentiate non-pathogenic yeasts from *Candida albicans* may result in an erroneous diagnosis of a mycosis, or lead to misconceptions regarding the incidence and significance of the pathogen in such areas.

While identification of *Candida albicans* cultures is most reliably based on fermentation and utilization of sugars (Wickerham and Rettger, **1939**), these reactions are cumbersome to perform routinely and give exact results only if pure strains are used (Langeron and Vanbreuseghem, **1952**). Slide agglutination tests with immune sera (Christie and Morton, **1953**), and the airpouch injection method recently described by Hill and Gebhardt (**1956**), are similarly laborious and time-consuming to carry out.

In the routine laboratory, chlamydospore formation on special media is still the main criterion for rapid identification of *C. albicans*. Ideally, the medium should stimulate chlamydospore formation within 24 to 72 hours. It should be simple to prepare and should be transparent, so as to permit direct examination of plates inoculated by the slit method, without the necessity of making culture mounts or preparing special slide cultures (Fusillo *et al.*, **1952**).

However, there are strains of *C. albicans* which will not readily form chlamydospores on standard media like corn-meal agar (Anderson *et al.*, **1944**; Marples and Di Menna, **1952**; Haley and Stonerod, **1955**), or on rice infusion agar (Taschdjian, **1953**). The writer noted that roughly one-third of some sixty cultures isolated from oral thrush, from skin lesions, or from the feces of newborn infants consistently failed to form chlamydospores and/or mycelium on rice infusion agar, though other-

[1] This work was aided by a grant from the Squibb Institute for Medical Research, New Brunswick, New Jersey, and by the Jewish Philanthropic League of Brooklyn, New York.

wise typical as regards morphology in the lesions and fermentation reactions.

Results were not improved by addition of 1% peptone to the rice infusion, by substituting tap water for distilled water in order to provide trace metals, by adding yeast extract, or by using as a polysaccharide source "cream of wheat" or "cream of rice," both of which are "enriched" with vitamins B_1, B_2, niacin, iron, calcium, and phosphorus.

The use of the Dalmau technique—covering the slit inoculum with a sterile cover slip in order further to reduce oxygen tension—had no effect. The age of the culture, also, did not seem to be a factor. Primary isolates aged 24 to 48 hours proved just as capable or incapable of forming chlamdospores as did fresh transfers from stock cultures.

In view of this difficulty, an attempt was made to carry out a comparative study of various media recommended for the gross or microscopic identification of *Candida albicans*. At the same time, a new medium will be described.

MATERIAL AND METHOD

19 "recalcitrant" strains of *Candida albicans* were used. A "normal" strain, which had formed chlamydospores on rice infusion agar within 24 hours, was used as a control. In addition to these twenty strains of *C. albicans*, one stock strain each of *C. krusei, C. parakrusei, C. Guillermondi, C. stellatoidea, C. tropicalis*, and *C. pseudotropicalis* was grown on the media described and commented upon below.

1. NICKERSON'S MEDIUM (Ortho)

Supplied by the manufacturers in small tubes and recommended for direct isolation and identification of the genus *Candida*. *Candida* species show a brown or black surface growth.

Comment: All members of the genus *Candida*, as well as some bacteria and *Geotrichum* sp., yield dark growths on this medium. Further identification is necessary in order to avoid misinterpretation of every dark-colored growth as *Candida albicans*.

If the medium is melted down, resolidified without slanting, and used for a stab culture, some strains of *C. albicans* will form mycelium and chlamydospores anaerobically, which can be observed in culture mounts.

2. PEPTONE AGAR, modified by Morton (**1955**) after Weld (**1953**).

Composition: 1% peptone, 2% agar in tap water.

Distinctive features claimed: "Spidery" colonies of *C. albicans* on surface streak after 24 hours' incubation at 37° C, visible under a plate microscope.

Comment: "Spidery" growth was observed in only 3 out of 20 cultures of *C. albicans* after one week's incubation on this medium (see TABLE I).

3. BACTO CHLAMYDOSPORE AGAR (Difco) after Nickerson and Mankowski (**1953**)

The medium was prepared from the commercially available dehydrated product and from slants which were melted down, poured into plates, and inoculated by surface streak and by the slit method.[2]

Distinctive features claimed: Dark blue or black surface growth. Chlamydospores within 24 to 96 hours at 25° C.

Comment: Blue or black surface growth was obtained with all seven *Candida* species tested. *C. albicans* formed chlamydospores anaerobically, but direct observation of the slit culture is impeded by the deep blue color of the medium and involves considerable eye strain.

4. DEXTROSE-PEPTONE-TWEEN 80 AGAR after Seeliger (**1955**)

Composition: Dextrose 0.1%, Bacto peptone 1%, NaCl 0.5%, Tween 80 1%, agar 1%. (Originally recommended for stab cultures with 0.4% agar.) Used here in plates, inoculated by slit method.

Distinctive features claimed: Pronounced mycelium formation in *C. albicans, C. tropicalis, C. krusei, C. stellatoidea.* Chlamydospores in *C. albicans.*

Comment: Although mycelium production was abundant, no chlamydospores were seen in the 20 cultures of *C. albicans* tested on this medium, probably due to the presence of dextrose, which inhibits their formation.

Profuse mycelium was observed also in *C. parakrusei* and *C. pseudotropicalis* in addition to the above-named species. No mycelium was formed by *C. Guillermondi.*

5. CREAM OF RICE INFUSION—TWEEN 80 AGAR

Composition: "Cream of Rice" 10 gm, agar 10 gm, tap water 1000 ml, Tween 80 10 ml.

Preparation: Bring water to a boil, sprinkle in Cream of Rice; boil 30 seconds, let stand 30 seconds. Filter through cotton, restore volume of filtrate to 1000 ml, add agar and Tween 80; autoclave. Pour into plates; inoculate by slit method.

Distinctive features: Abundant mycelium and chlamydospore formation by *C. albicans* within 24 to 96 hours (see TABLE I).

[2] The writer is indebted to Difco Laboratories, Detroit, Michigan, for a supply of chlamydospore agar slants.

Comment: Without addition of Tween 80, this medium gave only slightly better results than rice infusion agar. With Tween 80 added, results were excellent. Presumably other polysaccharide sources may be substituted for "cream of rice." [3] The latter is recommended, however, since it does not require prolonged boiling or simmering.

None of the non-pathogenic Candidas tested formed chlamydospores on this medium.

TABLE I

MORPHOLOGY OF 19 "RECALCITRANT" AND ONE "NORMAL" STRAINS OF *C. albicans* ON VARIOUS MEDIA

Strain No.	From	Direct smear	Peptone agar, 1 week	Dextrose-peptone-Tween 80 agar	Bacto chlamydospore agar 24–96 hrs	Cream of Rice infusion agar	Cream of Rice Tween 80 agar
						24–96 hours	
12	thrush	+	o	M	/	o	M/C
318	mouth	o	o	M	M	o	M/C
384	thrush	+	+	M	M/C	o	M/C
454	thrush	+	o	M	M	o	M/C
594	thrush	+	o	M	o	o	M/C
685	thrush	+	o	M	o	o	M/C
726	thrush	+	o	M	o	M/C	/
811	thrush	+	o	M	o	o	M/C
846	thrush	+	o	M	/	o	M/C
908	feces	+	o	M	M/C	M/C	/
Control							
927	feces	o	o	M	o	o	M/C
971	thrush	+	+	contaminated	M/C	o	M/C
982	thrush	+	o	M	M	o	M/C
1015	skin	+	o	M	o	o	M/C
1036	feces	+	+	M	/	M	M/C
1060	vagina	+	o	M	/	M/C	/
1064	thrush	+	o	M	o	o	M/C
1087	thrush	+	o	M	M	o	M/C
1162	thrush	+	o	M	M	o	M/C
1176	anus	+	o	M	M/C	o	M/C
Total M/C				o	4/16	3/20	17/17
Total M only				19/20	5/16	1/20	o

M: Mycelium only. M/C: Mycelium and Chlamydospores. /: Not done. +: Distinctive features present. o: Distinctive features absent.

RESULTS

The results of growing the 19 "recalcitrant" strains and "normal" control of *C. albicans* on the media described above are shown in TABLE

[3] Rosenthal (personal communication) reports improved results upon addition of 1% Tween 80 to corn-meal agar.

I. All had yielded brown or black colonies on Nickerson's medium (Ortho), as had also the six non-pathogenic species tested.

Regarding chlamydospore formation, it will be noted that the best results were obtained on the Cream of Rice Infusion—Tween 80 Agar. All 17 strains of *C. albicans* tested sporulated readily on this medium, whereas they had previously formed neither mycelium nor chlamydospores despite repeated trials and modifications of media and techniques. In several cases the first filaments and chlamydospores were noted within 12 hours after inoculation, and in 10 of the 17 strains they were well developed within 24 hours. Six strains required 48 to 72 hours, and one formed chlamydospores after 96 hours.

Since conclusion of the comparative study presented here, some 30 additional strains of *C. albicans* have been identified on this medium, with uniformly good results. Organisms which failed to form chlamydospores could be shown by their fermentation and utilization of sugars to belong to other species of *Candida*.

DISCUSSION

It appears that no reliable medium for simultaneous isolation and gross identification of *Candida albicans* has as yet been devised, so that routine identification still rests on its microscopic morphology or fermentation reactions on special media.[4] According to Wickerham, all strains of the species *Candida albicans* are inherently able to form chlamydospores under the following conditions: 1. a medium poor in nutrients, especially fermentable sugars, 2. reduced oxygen tension, 3. an incubation temperature below 25° C.

However, it appears that other factors, also, influence chlamydospore formation in *C. albicans*, and that these do not consist in vitamin, mineral, or amino acid deficiency. This might explain why Bacto chlamydospore agar (Difco) which contains ammonium sulfate, monobasic potassium phosphate and biotin, did not prove markedly more effective in inducing sporulation than other media not thus "enriched."

The pronounced effect of Tween 80 (polyethylene ester of sorbitan monooleate) on filament and chlamydospore production remains still to be explained. Dubos and Davis (**1946**) have shown that Tween 80 strongly stimulates growth of tubercle bacilli *in vitro*, whereas growth was inhibited by Tween 20 and Tween 40 (monolaureate and monopalmitate derivatives of sorbitan). Seeliger found Tween 80 by far the

[4] Shapiro and others recently reported that *C. albicans* forms colonies of characteristic color and configuration on Littman's oxgall agar (**1956**).

most effective of the Tween series for stimulating mycelium formation in *C. albicans* and other Candidas. The observations made in the course of the present study imply that Tween 80 simultaneously inhibits cell division and stimulates cell growth, processes which, according to Nickerson (**1954**), are distinct from one another.

The fact that some strains of *C. albicans* seem to require the stimulation (or inhibition) provided by Tween 80, while others sporulate readily without such an addition to a simple polysaccharide medium, points to the existence of physiologic variants within the species. These variations do not seem to affect the pathogenicity of the organism. However, there are indications that "recalcitrant" strains are isolated more frequently from the mucous membranes than from other areas where they might be mistaken for *C. stellatoidea* and *vice versa* unless carefully identified.

While these problems need further investigation, the combination of "cream of rice" infusion, agar, and Tween 80 is recommended as a simply prepared and reliable medium for identifying *Candida albicans* in routine laboratory procedure.

SUMMARY

19 strains of *Candida albicans*, roughly one-third of strains isolated from oral thrush, skin lesions, or the feces of newborn infants, failed consistently to produce mycelium and chlamydospores on rice infusion agar, while the other two-thirds gave satisfactory results.

In an attempt at identification, the 19 "recalcitrant" strains, as well as 6 non-pathogenic *Candida* species, were grown on various media recommended for gross or microscopic identification of *C. albicans*.

The best results were obtained with a medium newly described here and prepared from "cream of rice," agar, and Tween 80 in tap water. All of 17 strains of *C. albicans* tested readily formed chlamydospores and mycelium on this medium. The non-pathogenic Candidas formed mycelium only.

It appears that neither vitamin, trace metal, mineral, or amino acid deficiency, nor the age of the culture are factors that inhibit chlamydospore formation in certain strains of *Candida albicans*.

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QUANTITATIVE STUDIES OF FUNGUS SPORES IN THE AIR [1]

S. M. PADY [2]

(WITH 3 FIGURES)

It has long been known that fungus spores are air-borne and their relation to the spread of fungi, particularly of pathogenic forms, is well established. Many studies have been made to determine the number and type of fungus spores in the atmosphere, most of these being qualitative, consisting chiefly of exposing nutrient plates and adhesive-coated slides to the air. The techniques used before 1941 were described in a review by Lambert's subcommittee (**3**) of the Committee on Aerobiology of the National Research Council. Since that time several new quantitative samplers have been described. The General Electric Duplex Electrostatic Bacterial Air Sampler was designed to draw a measured amount of air between electrostatic fields, the organisms being precipitated to the surface of agar plates (**15, 16**). Although designed primarily for indoor use for bacteria, it has been found satisfactory for studies of outdoor air-borne fungi (**14, 19**).

Another quantitative sampler was the slit sampler designed by Bourdillon, Lidwell, and Thomas (**1**) for bacterial studies. Air was drawn through a narrow slit and the organisms impinged on the agar surface of the plate which revolved below the slit opening. The concentration of organisms in the air was calculated from the volume of air sampled and number of colonies that grew in the plates. Kelly and Pady (**14**) used this sampler in outdoor work in determining the concentration of air-borne bacteria and fungi. It was discovered that this sampler was also satisfactory to study fungus spores of the air (**22**). Hirst (**9**) in England recently devised a new type of slit sampler for continuous sampling of the fungus spore population. The Hirst Spore Trap was designed to sample the air through a 24-hour period, the spores being deposited on a glass slide moving slowly past the slit opening. Gregory

[1] Contribution No. 481, serial No. 626, Department of Botany and Plant Pathology, Kansas Agr. Exp. Sta., Manhattan.

[2] The author appreciates the cooperation of Dr. A. B. Cardwell and Dr. S. E. Whitcomb of the Dept. of Physics, the use of facilities, and the interest and assistance of Duane A. Rittis in many phases of the investigation.

(**7**) described a portable volumetric spore trap which overcame the lack of portability and need for a power source in the Hirst sampler. By the use of a manually operated pump, air was evacuated from a reservoir drawing air through an impactor unit with a slit orifice 2 × 14 mm.

Pady (**17**) in 1954 described a sampler which used the sampling chamber and slit assembly of Bourdillon but incorporated several other features. This was described as the Pady-Rittis modification of the Bourdillon slit sampler and will be referred to as the Pady-Rittis sampler. This paper presents data obtained from sampling the air for fungus spores, using this sampler.

MATERIAL AND METHODS

When aerobiological studies were started in Kansas in 1953, it was decided to build a sampler that would overcome the disadvantages of the Bourdillon sampler, namely, separate heavy vacuum pump and general lack of portability. The essential feature of this sampler was a small pump attached directly to the side of the sampling chamber. The pump consisted of a small squirrel cage-type blower of 50 CFM capacity. This eliminated the need for a large vacuum pump and made a compact light weight portable sampler possible (**17**). The sampler was designed to use 110 volts but a converter was added to permit its use on a car battery. Two slit openings were used, the size recommended by Bourdillon (0.4 × 27.5 mm) and a larger one (0.7 × 30 mm). Tests gave higher readings with the larger opening which was used throughout the two-year sampling period from June, 1953, to June, 1955. The air flow was calibrated with a displacement meter and determined to be 0.52 cu ft per min.

Glass slides lightly coated with silicone grease (DC-4) were exposed 30 minutes, tests having shown that this exposure gave a satisfactory deposit. Lactophenol with cotton blue and a cover glass were added to the slide and the deposit examined under high power. Since the spores were concentrated in a narrow band on the slide, reading the spores in a number of fields was comparatively simple. A field is the area covered by one sweep of the high power lens across the deposit. The total number of spores in the field was recorded, as well as the number of recognizable spore types. Hyphae and pollen grains were recorded also. Usually 20 fields were examined in detail which represented 42% of the total deposit. Calculations were then made for the numbers of spores per cubic foot and for such spore types as were present in quantity.

Samples were taken daily or weekly, usually between 9 and 10:30 A.M. from June, 1953, to June, 1955. The sampler was mounted on

the roof of Willard Hall on the Kansas State College sampus, approximately 150 feet above the ground. During June, 1953, several samples were taken daily, but from July, 1953, on, only one was taken per day, and during the winter one or two weekly. Two hundred fifty-six samples were taken during the two-year period.

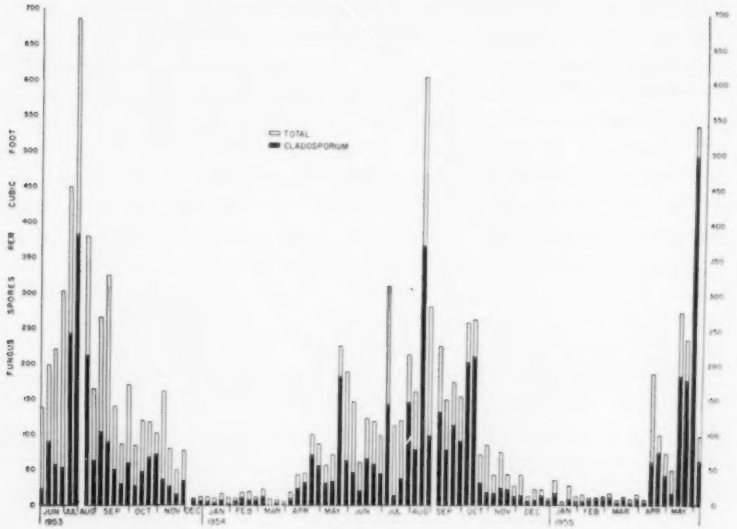
FUNGUS SPORES PER CUBIC FOOT
TOTAL
CLADOSPORIUM
JUN JUL AUG SEP OCT NOV DEC JAN FEB MAR APR MAY JUN JUL AUG SEP OCT NOV DEC JAN FEB MAR APR MAY
1953
1954
1955

FIG. 1. Weekly averages of total numbers of fungus spores and of *Cladosporium* obtained from 30 min. exposures of slides in Pady-Rittis sampler from roof of Willard Hall, Kansas State College campus.

RESULTS

Fungus spores were found on all the 256 slides that were exposed during the two year period. The growing season at Manhattan is 172 days on the average, with the last frost April 23 and the first Oct. 12, the season being extended by about 20 days in southern Kansas and decreased by 7 days along the Nebraska border. According to Cardwell and Flora (2), after the middle of February there is an irregular but general warming up until late July or early August, followed by a gradual decline. The number of fungus spores in the air corresponds closely with this seasonal pattern, increasing as growth begins in April, reaching a peak in July and August, and declining through September and October (FIG. 1). This pattern was evident in the 3 seasons sampled,

although numbers in any one month may be higher or lower than for the corresponding month of another year. For example, June, 1953, and May, 1955, had higher numbers than May and June, 1954. The fungus spore load from April to October, as averaged weekly, ranged from 50–700/cu ft with considerable daily variation. On July 7, 1953, a maximum reading of 1363.8/cu ft was obtained, while on July 19, 1954, an unusually low reading, 23.7 spores/cu ft was recorded. From December to March spore numbers were low, ranging from 5 to 20/cu ft, on a weekly average (FIG. 1). The lowest daily reading was 4.8/cu ft on March 23, 1954, the highest 35.7/cu ft on Jan. 4, 1955.

Cladosporium was the commonest fungus present, usually comprising a majority of the total spores present (FIG. 1). Its occurrence is definitely seasonal, with a high degree of variability in numbers from day to day during the summer months. During the growing season numbers ranged from 25 to 350/cu ft averaged weekly, with days when numbers extended much higher (to 1080/cu ft July 7, 1953) and much lower (3.9/cu ft July 16, 1954). In winter, numbers were low but some *Cladosporium* spores were always present, with numbers ranging from less than 1 to 15/cu ft, occasionally higher (18.6/cu ft Dec. 21, 1954) and often lower (0.9 Jan. 11, 1955, 0.6 Dec. 24, 1953, and a single spore Mar. 12, 1954). *Cladosporium* tends to occur in clusters or clumps of a few to as many as 60. Occasionally a complete branching conidiophore with chains of conidia almost intact will be observed.

Smut chlamydospores were surprisingly abundant, occurring throughout the year even in winter but with highest numbers during the growing season (FIG. 2). The peak of smut spore concentration seemed to occur during the last of June and first of July at the time when harvesting in the state was at its maximum. During this period spore concentrations were highly variable, ranging from 3 to 50/cu ft on the average, and on occasional days much higher with 182.4/cu ft Sept. 12, 1953, and 414.9/cu ft July 7, 1954. The weekly mean (FIG. 2) does not indicate daily fluctuations. For example, during the week of July 6, 1954, exposures taken July 6, 7, 8, and 9 had smut spore numbers of 3.9, 414.9, 2.7, and 4.2/cu ft, respectively. The smut spore shower of July 7 was due to a cold front which came in with strong winds. This air had passed over Nebraska where harvest was in progress and the smut spores probably originated there. Rain occurred as the front went through and presumably most of the spores were removed, as the following day the smut count was again very low. The weather July 8 was clear, rather cool, with light variable winds. In the winter chlamydospores were present on all but a few slides, ranging generally from 1 to 10/cu ft.

Chlamydospores were chiefly of the *Ustilago* type. The *Ustilago* spores were small, generally about 6 μ, thick walled, brown, often with a thinner area on the wall, and usually smooth. These appeared to be chiefly *U. hordei,* the cause of covered smut of oats and barley. Some of the larger spores of this type probably are referable to *U. tritici,* the common loose smut of wheat. Chlamydospores of corn smut, *U. maydis,* were common throughout the year but never in large numbers. Chlamydospores of *Sphacelotheca sorghi* are indistinguishable from those of *U. hordei* and since kernel smut of sorghum is common in the state, some of the spores may have been this species. Chlamydospores of *Tilletia* were not common, as indicated by their occurrence as single

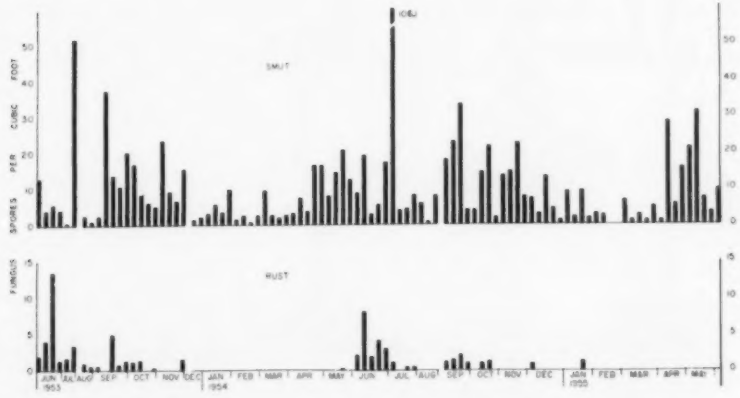
SMUT
SPORES PER CUBIC FOOT
RUST
FUNGUS
JUN JUL AUG SEP OCT NOV DEC JAN FEB MAR APR MAY JUN JUL AUG SEP OCT NOV DEC JAN FEB MAR APR MAY
1953
1954
1955

FIG 2. Smut chlamydospores and rust urediniospores from 30 min. exposures in Pady-Rittis sampler.

spores on 3 occasions, June 8, 1953, May 25, and Sept. 13, 1954. This is not surprising in view of the fact that covered smut or bunt is rare in the state.

Puccinia urediniospores were present in the air chiefly in June and September but occasionally in other months (FIG. 2). Spores of stem rust (*P. graminis* f. sp. *tritici*) and leaf rust of wheat [*P. recondita* (*P. rubigovera* f. sp. *tritici*)] were both present on the slides. The numbers given in FIG. 2 represent the weekly averages of the combined totals of all rusts present. Rust spores were present only on certain days and daily numbers varied from 1.2 to a maximum of 12/cu ft. June 16, 1954, some of the stem rust spores were observed to have germinated. The exposure was made with a north wind which had come in 12 hours

earlier as a cool front. At this date stem rust and leaf rust were abundant in wheat fields in Kansas (18) and the spore load probably came from fields of infected wheat in northern Kansas. The spores apparently had germinated during transit as no rain fell in Manhattan but some had fallen in areas to the north. Teliospores of *Puccinia* and *Uromyces* have been found on one or two occasions. Other urediniospores were present in small numbers but were not identifiable; it is

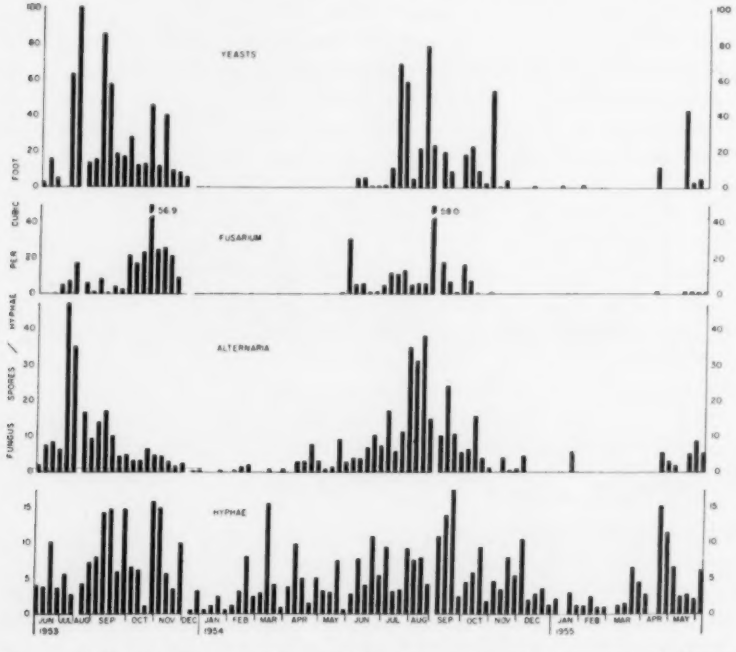

FIG. 3. *Alternaria* and *Fusarium* conidia, yeast cells, and fragments of fungus hyphae from 30 min. exposures in Pady-Rittis sampler.

believed, on the basis of their occurrence in August and September when wheat rusts are normally not present in large numbers in the state, that these were spores of grass rusts.

Alternaria spores were the most conspicuous objects on the slides because of their size, dark color, and their abundance during the growing season (FIG. 3). They had a weekly range from 2 to 40/cu ft with occasional days when numbers were higher. The peak load was on July 7, 1953, when 97.8 spores/cu ft were recorded. During the winter,

Alternaria was almost completely absent, being present on only a few days; for example, in January, 1955, *Alternaria* was absent from all slides except on Jan. 18, when 5.7 spores/cu ft were recorded.

Yeast cells were present in concentrations up to 100/cu ft averaged weekly on most slides exposed during summer months (FIG. 3). Counts were difficult to make as there were many small hyaline single-celled bodies which may have been either yeasts or small spores. Those below 5 μ were not included in the counts, thus the yeast concentration as recorded is probably low. The variability in numbers is apparently due to the fact that yeasts increase greatly following rains, and tend to be low or even absent during hot dry periods in summer. Aug. 9, 1954, yeasts were 12+/cu ft in a total spore load of 450.8/cu ft. The following day the total number of all spores and cells was 594/cu ft, and the yeasts were so numerous that a count could not be made, but estimates would be at least 60 yeast cells/cu ft. August 11 a similar situation prevailed and again yeasts were estimated at 30/cu ft. An accurate count was obtained Aug. 12 and it was found that yeast cells were 211.2/cu ft out of a total count of 490.5/cu ft. August 10 to 12 the weather picture was as follows: August 10 and 11 winds were easterly and precipitation was recorded here on Aug. 11 and also in eastern Kansas, which caused considerable reduction.

Fusarium spores were also seasonal in their occurrence, with weekly averages from June to October under 25 spores/cu ft except on 3 occasions. A high degree of daily variation occurred, as, for example, on Sept. 7, 8, 9, 1954, when readings were 0, 0 and 79.5/cu ft. Numbers over 50/cu ft occurred on 4 days, July 23/54, Sept. 9/54, Oct. 23/53 and Aug. 18/54 with concentrations of 57.6, 79.5, 87.5, and 162/cu ft. There is evidence that *Fusarium* increases following precipitation. Sept. 8/54 was hot with strong southerly winds. That evening rains occurred as a cool front passed through and Sept. 9 was cool with northerly winds. The spore concentration on these two days was 2.4 and 79.5/cu ft. A similar situation occurred Oct. 20–22, 1953, when general rains fell in the North Central states, including Manhattan. Oct. 22 winds were northerly and in the sample made on this date *Fusarium* had a concentration of 32.1/cu ft. Northerly winds continued through Oct. 23 and on that day after at least 30 hours of north wind the count reached 87.5 *Fusarium* spores/cu ft.

The following fungus spores were present on some of the slides exposed during the growing season but in such low numbers that they were not calculated on a cubic foot basis: *Helminthosporium, Cercospora, Penicillium* (or *Aspergillus*), *Septoria, Stemphylium, Sporormia, Pleo-*

spora, Venturia-like, *Leptosphaeria*-like, and *Curvularia*. On a few occasions *Erysiphe, Heterosporium, Nigrospora*-like, *Cephalothecium, Monilia*-like chains, and *Sordaria*-like spores were present. Basidiospores were present, occasionally in high numbers, from June to September following rains. Ascospores were frequently present, as indicated above, with a complete ascus and spores in some cases.

Unidentified spores were present on all slides, particularly in the 1-celled group. Detailed records were made of the principal types of unidentified spores. Since this study involved 256 exposures, it is difficult to present all of the information on the many spore types found. Accordingly, it was decided to select a few samples taken in summer and present a complete analysis of what is believed to be typical conditions. This is presented in TABLE I.

It should be noted that spores of some 15 genera, 4 other categories of spore types, hyphae, and pollen are also included in this table. July 1, *Cladosporium, Alternaria,* yeasts, and hyaline basidiospores were the most abundant types. The basidiospores were large, thin walled, with a prominent apiculus, and were presumed to be mushroom spores. Early July 2 a cool front passed through and the exposure on that day sampled northern air. The total number of spores was greater and most of the fungi, except *Alternaria,* showed an increase and included a smut spore shower (207/cu ft). Rain of 1.29 inches July 3 preceding the exposure reduced the total markedly, and *Cladosporium, Alternaria,* and *Ustilago* were almost completely absent, whereas yeasts, basidiospores, and *Venturia*-like spores were in greater concentration.

The exposure July 6 was made following 2 hot days with maxima of 101° and 102° F, respectively. A cold front came through July 5, bringing rain, north winds, and lower temperatures with these conditions continuing into the following day. The sample July 6 indicates a mixture of *Cladosporium,* usually characteristic of warm dry air, and yeast, basidiospores, and *Fusarium,* characteristic of moist cooler conditions. *Stemphylium,* which is considered to be a cool weather type, was also present. The northeastern wind continued into July 7, bringing in much dust and a high spore load with very high numbers of *Cladosporium, Alternaria,* basidiospores, and yeasts. The following day spore numbers were greatly reduced, but the constituent population was similar to that of July 7. The sample July 9 had higher numbers with another smut spore shower, but with yeasts absent. Numbers July 10 were low, and except for *Cladosporium,* spores were few in number and type.

Occasionally chains of bacteria were observed, particularly when they absorbed the cotton blue stain, which made them easily visible under the

TABLE I

ANALYSIS OF FUNGUS SPORES PER CUBIC FOOT ON 8 SLIDES EXPOSED 30 MIN., ALL AT APPROXIMATELY 9:00 A.M., JULY 1953, FROM ROOF OF WILLARD HALL, KANSAS STATE COLLEGE, MANHATTAN. Numbers in parentheses are total numbers present.

	July							
	1	2	3	6	7	8	9	10
Total spores/cu ft[1]	363	597	273	548+	1363+	390	803	321
Cladosporium	182	171	1	182	1080	144	310	234
Alternaria	53	16	—	16	98	22	25	16
Ustilago	0.6	207	—	18	12	36	180	13
Puccinia	2	5	—	—	—	2	7	1
Hyaline basidiospores	22	30	75	64	72	40	60	6
Venturia-like	7	47	38	8	—	—	—	—
Fusarium	0.6	16	14	20	13	36	17	1
Septoria	2	6	1	7	1	4	—	—
Helminthosporium	(1)	—	—	(1)	3	2	(2)	—
Yeasts[2]	60	54	108	120+	[3]	83	—	—
Brown, *Fusarium*-like	—	13	5	12	5	2	1	—
Cercospora	1	(1)	—	(1)	5	(1)	1	(2)
Hyphae	14	4	—	4	1	3	4	2
Large purple spores	(3)	—	—	—	—	—	—	—
Streptomyces	[4]	—	—	—	—	—	—	—
Pleospora	—	—	3	—	—	—	—	—
Penicillium or *Aspergillus*	—	—	—	(10)[5]	—	(15)[5]	—	—
Sporormia	—	—	—	(1)	—	—	—	—
Brown basidiospores	—	—	—	20	28	3	—	(3)
Stemphylium	—	—	—	(2)	—	—	2	—
Pollen	—	—	—	—	—	1	1	—
Temp. at 9:00 A.M. ° F	86	80	70	67	74	75	72	73
Wind	S/17	NE/9	S/5	S/NE/10	E/NE/7	E	NE/3	S/E/2
Duration	3 days	few hrs	8 hrs	14 hrs	24 hrs	8 hrs	32 hrs	
Precip.		T	1.29	.13				
Remarks			38 hrs after rain	14 hrs after rain	Much dust			

[1] Many small oval spores not counted.
[2] Difficult to identify; many small single cells included.
[3] Too many to count.
[4] An entire colony, with filaments and numerous coiled spores.
[5] In one group.

high-power magnification used. One such case occurred in the July 2 sample. An interesting discovery was the presence of a microscopic colony of *Streptomyces* complete with several coiled chains of spores in the sample July 1 (TABLE I).

To illustrate the winter spore picture, 6 exposures, taken on successive days, are analyzed in TABLE II. Spores were still present in the winter air, but numbers and types were greatly reduced, compared with

summer conditions. The exposure Dec. 21 with a total of 9 spores/cu ft was typical of the winter fungus flora. *Cladosporium*, *Alternaria*, and *Ustilago* were the principal spores present. Small brown 1-celled spores were fairly abundant; it is not known if these are typical of winter conditions. They may also be present in summer but obscured by dust and debris. From Dec. 18 to 21 the wind was southerly, changing to northerly only 8 hours before the exposure was made. This situation con-

TABLE II

ANALYSIS OF FUNGUS SPORES PER CUBIC FOOT ON 8 SLIDES EXPOSED 30 MIN., ALL AT APPROXIMATELY 9:00 A.M., DECEMBER 1953, FROM ROOF OF WILLARD HALL, KANSAS STATE COLLEGE, MANHATTAN. Numbers in parentheses are total numbers present on slides.

	December					
	21	22	23	24	28	29
Total	9	13	13	5	9	27
Cladosporium	2	5	7	0.6	4	10
Alternaria	—	1	1	(1)	(1)	3
Ustilago	0.6	2	2	1	1	6
Hyphae	0.6	2	(1)	—	1	7
Small brown	4	(1)	1	1	(2)	1
Venturia-like	—	(2)	—	—	—	—
Small hyaline	—	2	—	—	(1)	—
Penicillium or *Aspergillus*	(2)	—	3	1	2	2
Yeast	—	—	—	—	—	(1)
Septoria	—	—	—	—	—	(1)
Temp. °F	40	8	16	26	28	34
Wind	N/2	N/18	SW	W/SW/3	SW	W/SW
Duration	8 hrs	32 hrs		24 hrs	3 days	24 hrs
Precipitation		3 in. snow in afternoon				
Remarks		no dust	much dust	dust		much dust

tinued into Dec. 22 and the spore picture is similar to that of Dec. 21. The following day winds again were from the south, bringing considerable dust and some increase in total spore numbers. Although the wind was unchanged Dec. 24, numbers were unaccountably lower and many fields had no spores whatsoever. Average winter conditions are indicated in the readings of Dec. 28, whereas the next day numbers had increased and considerable dust was present.

Hyphal fragments were so common and abundant that counts were made, although they were not included in the total spore number. On a weekly average they ranged from 2 to 15/cu ft without seasonal peaks (FIG. 3). Occasionally, fairly high numbers were found in the winter,

as, for example, Jan. 20, 1954, with strong cold winds (N/20, 8° F), the concentration was 4.8/cu ft, and Mar. 12, 1954, with warm southerly winds (S/SE/10, 55° F), there were 15.6 hyphae/cu ft. There was some indication that the fragments were more abundant when the winds were strong. Sept. 12, 1953, with brisk northerly winds, a peak of 52.8 hyphae/cu ft was reached, while on successive days the numbers were 22, 14, 14, and 21/cu ft. The lowest reading (5.1/cu ft) of fungus spores was recorded Jan. 11, 1955, yet even here hyphae were present (0.6/cu ft).

Most hyphal fragments were brown and thick walled, many of them consisting of the broken terminal portion of conidiophores. Occasionally a complete cluster of several radiating conidiophores of the *Alternaria* type was found. Hyaline hyphae were either less numerous or were not recognized, although one case was recorded of a fragment of hypha bearing a clamp connection. Small sections of brown-celled hyphae resembling *Pullularia* were occasionally found as were microscopic rhizomorphic strands. Small irregular clumps of enlarged cells and hyphae were sometimes encountered, particularly on small portions of plant debris. Since most of the fungi in the air are considered to be soil types, it is not surprising that the strong winds of the Great Plains carry hyphae, as their scarifying action would serve to set hyphae free.

DISCUSSION

Seasonal distribution of the fungus spore population has been observed by many authors (**4, 5, 8, 11, 23**). In a year round study of the fungi of the air, using both slides and nutrient plates, the author found that the numbers of fungi were highest during the growing season and were very low, although not completely absent, from the air in winter in Montreal, Canada (**21**). The fungus spore population in Kansas is similar to that reported in Montreal except that numbers are considerably higher. Since most of the spores caught on the slides are considered to have come from fungi in the soil, it may be assumed that the longer growing season and generally milder climate of Kansas were more favorable for the growth of soil fungi than Canada. During the sampling period there were several periods of drouth which probably affected the spore count, as a weather change during the growing season which brought in air from regions where rain had fallen usually resulted in a substantial increase in spore numbers. Another factor that bears on the spore load carried is the intensity of the wind, high winds usually carrying more spores than air masses with lower velocity. That such

winds are effective in liberating soil fungi is reflected in the year-round presence of hyphal fragments in the air.

Since previous studies in Canada (**13**), in the Arctic (**22**), and over the Atlantic Ocean (**20**) had indicated high spore loads for tropical air and low spore loads for polar air masses, it was expected that the same would hold true for Kansas. Such, however, was not the case. Polar air masses are usually modified by the time they reach this state and have had an opportunity to pick up spores as they passed over the agricultural lands of the western Canadian provinces, the Dakotas, Nebraska, and northern Kansas. Polar air may thus carry higher numbers than tropical air masses. No attempt has been made in this paper to identify the air masses, because only one 30-minute sample was made daily. It would appear that hot dry winds carry a high proportion of *Cladosporium* spores, while cooler air, particularly following precipitation, is characterized by yeasts, basidiospores, and *Fusarium*. Similar findings were obtained by Gregory (**6**) and Hirst (**10**) in England.

Many unidentified fungus spores were present on the slides, ranging from very small hyaline or colored spores to large multicellular cells, often of unusual form. Culture work is planned for the future and it is anticipated that some of these fungi will respond to culture techniques. Viability studies are desirable also to determine the number of viable spores in the air. In the Arctic on a flight to Resolute Bay, the ratio of nonviable to viable spores was 27.3 to 1, while at Churchill it was 6.8 to 1 (**19**), and at Montreal it was 5–15 to 1 (**21**). Studies in the Great Plains provide an opportunity to determine the effects of hot dry winds on the viability of the air-borne fungus flora.

On several occasions 8 spores in a group were found, the arrangement of the spores being such as to suggest that they were ascospores. In a few cases a definite ascus with spores was observed. Hirst (**10**) found ascospores to be fairly common in the air over England, particularly during damp weather which seemed to favor their discharge. Ascospores were not common on the slides or were not recognized in the study reported here. Plant fragments such as wood cells, epidermal cells of leaves, and hairs were not uncommon and often had spores and hyphae associated with them. Hyphae in cells of detached leaf hairs were fairly common.

The year round presence of hyphal fragments, as well as their occasional high incidence, was rather surprising. Generally the hyphae were short, but long ones were also present. As indicated earlier, many of them were conidiophores and the majority were thick-walled, brown, and septate. It would be of interest to know if these fragments were

viable and would grow in culture. *Cladosporium* is the commonest fungus in the air and its spores are widely distributed (**5**, **10**, **12**, **23**). Frequently the spore clumps include portions of the conidiophores, and entire fruiting heads have been seen on the slides. Since viable spores have been found up to 10,000 feet in the Arctic (**19**) and over the Atlantic Ocean (**20**), it is evident that *Cladosporium* is a very resistant fungus, and it would be desirable to know if this characteristic is shared by the hyphae. In the event that hyphal fragments of fungi are found to be viable, it would throw further light on the ability of fungi to survive unfavorable conditions, particularly in view of their occurrence throughout the year and abundance at certain times (FIG. 3). Moreover, our present system of counting would need to be revised as hyphae are not at present included in the counts of air-borne fungi.

Data are needed on the spore loads at different times of the day. Hirst (**10**) has shown that there is a diurnal pattern in the spore population at Rothamsted, England, with peaks around noon and low numbers at night, *Cladosporium*, *Alternaria*, and rust spores having peaks at about 1 P.M., *Erysiphe* and *Ustilago* about 2–3 P.M. Basidiospores, on the other hand, showed just the opposite distribution, as did some ascospores, with peaks just after midnight. If this situation should be true for Kansas, the samples would then not represent the optimum as far as testing is concerned, as the exposures were usually made in midmorning. It is desirable that studies be made to determine if there is a diurnal periodicity in the numbers of fungus spores in the air in Kansas.

SUMMARY

Two hundred fifty-six silicone treated slides were exposed to the air in a Pady-Rittis sampler between June, 1953, and June, 1955, and the total number of fungus spores, as well as the commonest types were determined on a cubic foot basis. An exposure of 30 min. was made daily in summer and once or twice a week in winter. Fungus spores were present throughtout the year but were seasonal in their distribution with peaks in July and August and low concentrations in winter. Numbers in summer ranged from 50 to 700/cu ft with occasional peaks to over 1300/cu ft. In the winter numbers were usually 5 to 20/cu ft.

Cladosporium was present throughout the year, comprising the bulk of the spores in summer. Numbers in the summer were usually 25 to 350/cu ft, but higher concentrations were common, with one spore load 1080/cu ft July 7, 1953. *Ustilago* chlamydospores were also present all year with a peak in June at harvest time, ranging from 3 to 50/cu ft in the summer with occasional spore loads up to 415/cu ft. In the winter

numbers were from 1 to 10/cu ft. *Puccinia* urediniospores were found chiefly in June with high numbers on only a few days and in concentrations from 1.2 to 12/cu ft. *Alternaria* spores were seasonal in distribution with numbers ranging from 2 to 40/cu ft in the summer, with a maximum of 97.8/cu ft on July 7, 1953. Yeast, *Fusarium*, and basidiospores were definitely seasonal in their occurrence and appeared to be abundant following rains. Yeast cells were usually 1–40/cu ft with one high of 211/cu ft, whereas *Fusarium* spores were usually lower. Basidiospores were sporadic in appearance with numbers occasionally up to 75/cu ft.

Spores of the following were found occasionally on the slides: *Helminthosporium, Cercospora, Penicillium* (or *Aspergillus*), *Septoria, Stemphylium, Sporormia, Pleospora, Venturia*-like, *Leptosphaeria*-like, *Curvularia, Erysiphe, Heterosporium, Nigrospora*-like, *Cephalothecium, Monilia*-like chains and *Sordaria*-like. Unidentified spores, usually 1-celled, made up a large portion of the spore deposit. Hyphal fragments were present throughout the year without seasonal peaks, ranging from 2 to 15/cu ft and sometimes as high as 53/cu ft.

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RHIZOCTONIA SOLANI AS A PARASITE OF FUNGI [1]

EDWARD E. BUTLER [2, 3]

(WITH 3 FIGURES)

Rhizoctonia solani Kühn, an important soil-inhabiting facultative parasite, has an extremely wide host range. More than 200 species of flowering plants are host to this fungus (**10**) but little is known regarding the capacity of *R. solani* to parasitize organisms other than seed plants.

Butler and King (**3**) reported that unidentified isolates of *Rhizoctonia* parasitized fungi in addition to inciting lesions on the roots of strawberry (*Fragaria* spp.). It is the purpose of the present paper to report in more detail the results of studies on *R. solani* as a parasite of other fungi. These investigations include studies on host-parasite relationships, host range, parasitic specialization, mode of parasitism and the influence of certain environmental factors thought to be of importance to parasitism.

MATERIALS AND METHODS

Unless stated otherwise *Rhizoctonia solani* and a potential host fungus were grown on potato-dextrose agar (PDA) containing the aqueous extract from 300 g potatoes, 10 g glucose, 15 g agar and tap water to make one liter. The reaction after autoclaving was pH 6.7–7.0. Portions of the substrate containing mycelium of the host and parasite, respectively, from actively growing cultures were placed simultaneously on opposite sides of the agar in 250 ml flasks or in Petri dishes, if so stated, and incubated at approximately 25° C. Forty-eight to 72 hours after contact of the two colonies, a minimum of three samples of mycelium were removed from areas of the host colony overgrown by the parasite and examined for evidences of parasitism. Each sample contained more than 100 hyphae of a potential host.

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[2] Part of a thesis presented to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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Isolates of *R. solani* employed in these investigations were diverse culturally and morphologically; no two isolates were culturally alike. The intensity of production and form of sclerotia were conspicuously variable. Sclerotial initials of the highly virulent isolates R-1 and R-2 were comprised of more or less globose cells whereas other isolates formed initials from typical doliform cells. Under special conditions cultures R-5 and R-6 (TABLE II) produced basidiospores and were identified as *Pellicularia filamentosa* (Pat.) Rogers. Each of the isolates of *R. solani* pathogenic to fungi had previously been shown to attack cultivated plants.

The general account of host-parasite relations, with cited exceptions, is based on the susceptible or very susceptible species given in TABLE I and the highly virulent isolates R-1, R-2, and R-4 of *R. solani*.

OBSERVATIONS ON HOST-PARASITE RELATIONSHIPS

If *Rhizoctonia solani* and a susceptible fungus host are grown together on potato-dextrose agar or other suitable substrate, the parasite is stimulated to form a zone of dense mycelium at the juncture of the two colonies. This zone enlarges with the advance of the parasite and ultimately extends through the host colony. Infection of the host is first observed near the point of initial contact of the two colonies. A few infected hyphae may be detected 24 hours after contact but maximum infection generally occurs at 48 to 72 hours. Radial extension of the host mycelium does not cease abruptly upon contact with the parasite but continues to grow a limited distance into the advancing colony of *R. solani*. This is especially true for certain mucoraceous hosts. *Rhizopus nigricans* Ehrenberg, for example, may spread over much of the substrate in a flask, because of its rapid surface and aerial growth, before it becomes extensively parasitized.

Modes of attack by the parasite. *R. solani* parasitizes other fungi by penetrating their aerial hyphae and sporophores (FIG. 2, C; FIG. 3, E) and establishing an internal parasitic mycelium or by coiling around the aerial parts (FIG. 2, A, E) or by a combination of both (FIG. 2, B). These modes of attack can be resolved into two principal types: the coiling type in which the parasite only coils around the host hyphae, and the penetration-coiling type in which the parasite always penetrates the host but may also coil around the host parts. Species of *Pythium* (TABLE I) are parasitized by the first method and species of the Mucorales and the hyphomycete *Ambylosporium botrytis* Fres. by the latter.

Penetration of a host is effected by infection hyphae 1–2μ in diameter (FIG. 3, E). These hyphae may arise from the advancing tip of iso-

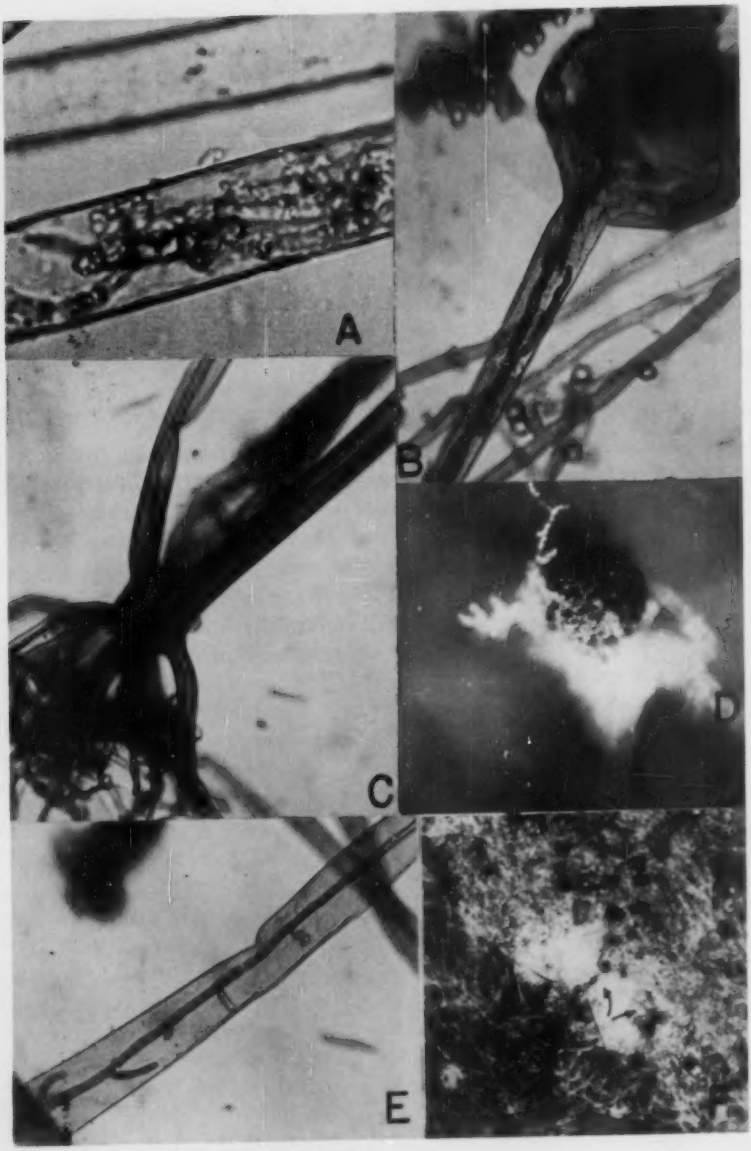

FIG. 1, A–F.

lated hyphae, which contact the host; from the advancing tip of hyphae growing parallel in contact with the host (FIG. 3, E) or from hyphal loops or coils of the parasite (FIG. 2, C).

The basic mode of attack depends primarily upon the genotype of the host and parasite. Although changes in the environment may alter the intensity of infection, it has not been possible, for example, to induce *R. solani* to parasitize *Pythium debaryanum* Hesse by penetrating its hyphae nor to induce *R. solani* to attack *R. nigricans* solely by the formation of parasitic coils.

Effects of parasitism on host and parasite. The general visible effects of parasitism are directly related to the modes of attack by the parasite. The effects resulting from the penetration-coiling type will be considered first.

After penetrating the host wall the parasite grows rapidly within the host where it branches and enlarges (FIG. 3, A) to fill a portion of the mycelium. An extensive internal network of parasitic mycelium results from multiple infection of the host. The parasite replaces and apparently utilizes the protoplasm of the host as it multiplies. Internal hyphae grow outward and add to the profusion of hyphal coils already present. As a rule the internal hyphae remain within the host. The parasite also coils profusely around the host hyphae and sporophores; numerous infection hyphae arise from these coils. However, in the reaction between *Syncephalastrum* sp. and *R. solani* the parasitic hyphae are strictly internal. Young, rapidly growing host hyphae are most susceptible to attack. As a result of parasitism the host wall may collapse (FIG. 2, F) but it has not been observed to disintegrate.

In susceptible species of *Rhizopus* and *Mucor* (TABLE I) a dense layer of parasitic hyphae is formed around many of the developing sporangia (FIG. 1, D, F, lower left). These hyphae penetrate the sporangial wall and ramify between the spores, if present. In *Mucor recurvus* Butler the spores of some of the sporangia thus attacked are morphologically abnormal, ranging in size from 12 to 22 μ in the long axis and appearing round, oval, and amoeboid. Normal spores are elliptical and 20 to 26 μ in length.

R. solani destroys much of the aerial growth of a host but does not completely kill cultures of even the most susceptible Mucorales. Trans-

FIG. 1. *Rhizoctonia solani* parasitizing *Rhizopus* sp. A. Disorganization of hyphae of *R. solani* within a stolon, × 500. B, C, E. Same, × 300, × 200, × 220, respectively. D. A tuft of sclerotial cells emerging from the base of a sporangium, × 110. F. A culture severely attacked by *R. solani*. Arrows indicate collapsed and overgrown sporangiophores following severe infection.

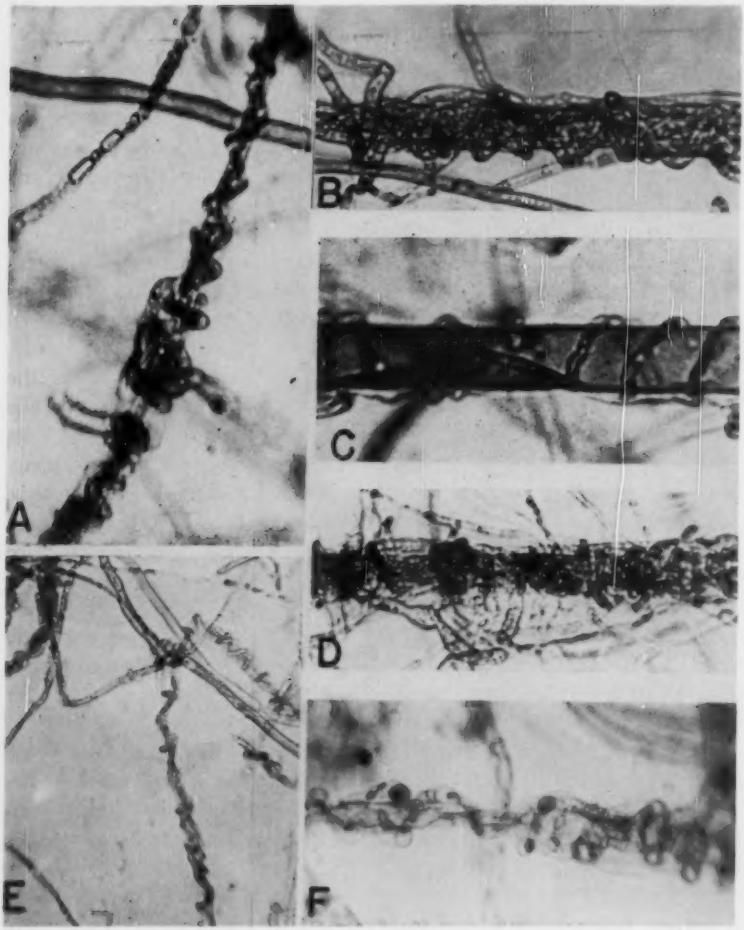

FIG. 2. *Rhizoctonia solani* parasitizing various fungi. A, E. Hyphae of *Pythium debaryanum* within hyphal coils of *R. solani*, × 270 and × 100, respectively. B, D. Portions of sporangiophores of *Rhizopus nigricans* completely filled and enveloped by hyphae of the parasite, × 330 and × 240, respectively. C. Portion of a sporangiophore of *R. nigricans*, × 300. Upper center shows apparently unsuccessful attempt of *R. solani* to penetrate the sporangiophore wall. A similar attempt is shown diagrammatically in FIG. 3, E. F. Portion of a sporangiophore of *Mucor recurvus* within coils of *R. solani*. Parasite beginning lysis, × 270.

fers of substrate hyphae and spores following a vigorous attack by the parasite readily establish new cultures of a host.

A host may resist infection by *R. solani.* Two kinds of host resistance have been observed: lysis of the parasitic hyphae (FIG. 1, A) and the walling-off of infection filaments (FIG. 3, E). In the relationship between *R. solani,* isolate R-5, and *R. nigricans,* some hyphae of

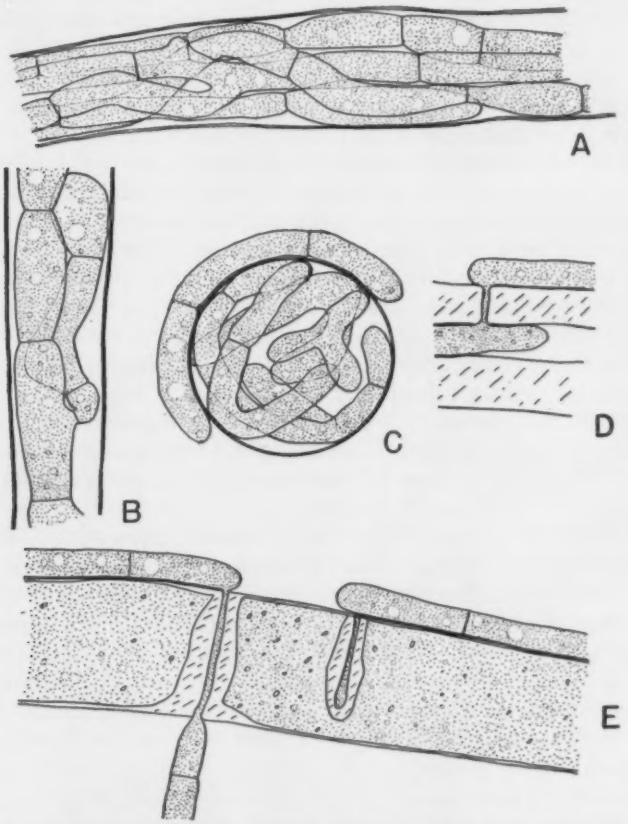

FIG. 3. Semi-diagrammatic drawings of penetration and internal development of *Rhizoctonia solani.* A, B. Portions of sporangiophores of *Mucor recurvus*—sporangiophore walls distended by the parasite. C. A conidium of *Pythium splendens.* D. Portion of a lint hair of cotton (*Gossypium* sp.). E. Infection hyphae of *R. solani* walled-off by a stolon of *Rhizopus nigricans.* All drawings approximately × 600.

the parasite break down almost immediately after entry into the host (FIG. 1, A). In this case the invading hyphae are actively eliminated by the host protoplasm. In all other relationships observed by the writer lysis seems ultimately to occur but is delayed until maximum infection has been reached and the parasite has grown extensively within or around the host.

Infection hyphae are frequently walled-off by a protective sheath of wall-like material (FIG. 3, E). In *M. recurvus* this sheath and the walls of the young sporangiophores take on a light blue to purple color when stained with zinc-chloriodide. Such a "mechanical" means of resistance is frequently observed in *R. nigricans, M. recurvus* and *Phycomyces blakesleanus* Burgeff.

In the coiling type of host-parasite relationship, the visible effects on host and parasite are not as intricate as those previously described for the Mucorales. *R. solani* overgrows the host colony and forms tight coils around the host hyphae. The protoplasm of the encircled hyphae either coagulates (as in upper left FIG. 2, A) into oil-like globules or becomes progressively less dense and disappears. The dilution effect on the host protoplasm may be due, in part, to migration of the protoplasm away from the site of infection. The walls of the host hyphae collapse but seem to persist. Infrequently the hyphae of *R. solani* penetrate the hyphae of the species of *Pythium* but once inside develop feebly. *Pythium splendens* Subram. is the exception; hyphae of *R. solani* penetrate the walls of the large terminal conidia (FIG. 3, C) and fill them with masses of tightly packed hyphae.

Transfers of agar containing the hyphae of *Pythium butleri* Subram. and *R. solani* to fresh media yielded subcultures of *R. solani* only, while subcultures of the other *Pythium* species given in TABLE I gave both the *Pythium* species and *R. solani.* Failure to obtain *P. butleri* after the colony was overgrown with the parasite might indicate the existence of lethal substances produced by *R. solani.* However, other observations did not confirm this hypothesis. Autolysis occurred normally in cultures of *P. butleri* on PDA at 25° C after 4–5 days but cultures were not dead. In the presence of *R. solani* autolysis was accelerated and coagulation of the protoplasm and subsequent collapse of the aerial hyphae not in contact with the parasite took place in from 48 to 72 hours.

INDEX OF HOST SUSCEPTIBILITY

In order to evaluate and compare the influence of various factors affecting the reaction between a host and the parasite, an attempt was made to provide an index of host susceptibility. The types of host reac-

tion, defined for the penetration-coiling modes of attack by *Rhizoctonia solani* as previously described, are as follows:

Type 0—Immune. No parasitic interaction between the parasite and a potential host.

Type 1—Resistant. Susceptible hyphae distributed sparsely throughout the portion of the host colony overgrown by *R. solani.* The internal parasitic mycelium limited to short, generally unbranched or once-branched filaments (FIG. 1, E). Coiling by the parasite may occur but is weakly developed.

Type 2—Slightly susceptible. Infected parts more prevalent than in Type 1. Internal parasitic mycelium developed beyond the single filament stage of Type 1, but usually not multiplying to fill the infected host part. Coiling of the parasitic hyphae generally absent. If present, not vigorous and sparsely distributed.

Type 3—Susceptible. Infection centers abundant. Internal parasitic mycelium well-developed within the host (FIG. 3, A) (FIG. 2, B). Coiling by the parasite vigorous and widespread.

Type 4—Very susceptible. Similar to Type 3, but a more intense host-parasite interaction. Infection hyphae and hyphal coils formed profusely by the parasite (FIG. 2, D), affecting nearly all of the host mycelium.

The reaction of members of the Peronosporales to *R. solani* was recorded using the numerical system and type designation described for the *Mucor* type hosts. The frequency and intensity of parasitic hyphal coils were used as criteria for assessing the type of host reaction.

Although the distinction between a resistant and a susceptible reaction can be made readily, it is considerably more difficult to distinguish between a resistant (Type 1) and a slightly-susceptible (Type 2) and between a susceptible (Type 3) and a very-susceptible (Type 4) reaction. Hence, the discrimination between closely related types is clearly a matter of judgment, and the effect of a given factor on the parasitic processes can be significantly evaluated only by widely separated types of host reaction.

FUNGUS HOST RANGE

Several fungi representing the classes Phycomycetes, Ascomycetes, Basidiomycetes and Fungi Imperfecti were tested as possible hosts for *Rhizoctonia solani.* Most of the species are known to inhabit soil and may live in association with the parasite. The host range and relative susceptibility of each species is summarized in TABLE I.

Except for *Ambylosporium botrytis* of the Fungi Imperfecti, only

Phycomycetes of the orders Mucorales and Peronosporales were parasitized. There were differences in susceptibility to *R. solani* between genera and between species in the same genus. Three species of *Pythium* were susceptible, two slightly susceptible, and three immune to *R. solani*, two species of *Mucor* were susceptible and one immune. The (+) and (−) strains of *Rhizopus nigricans* and *Phycomyces blakesleanus*, respectively, were equally susceptible to *R. solani*. The homothallic Mucorales, *Mucor genevensis* Lendner and *Zygorhynchus* sp. were immune.

The reason for the apparently narrow range of fungous hosts is not clear. Members of the orders Mucorales and Peronosporales have been recorded a number of times as hosts to other fungi; as a group they seem particularly susceptible to attack. Raybaud (**9**) described the behavior of a species of *Fusarium* which attacked only the young, densely

TABLE I

SUSCEPTIBILITY OF FUNGI TO ISOLATES R-1, R-2, and R-4 OF RHIZOCTONIA SOLANI

Fungi tested as hosts	Host reaction[1]
PHYCOMYCETES (Mucorales)	
Rhizopus nigricans Ehrenb. (+) and (−) strains	4
Rhizopus sp.	4
Syncephalastrum sp.	4
Mucor recurvus E. E. Butler	3
Mucor flavus Bain.	3
Helicostylum sp.	3
Cunninghamella echinata Pispek	2
Phycomyces blakesleanus Burgeff (+) and (−) strains	1
Mucor genevensis Lendner	0
Circinella simplex Van Tiegh.	0
Thamnidium sp.	0
Zygorhynchus sp.	0
(Peronosporales)	
Pythium splendens Braun	4
P. butleri Subram.	3
P. debaryanum Hesse	3
P. irregulare Buisman	2
P. vexans deBary	2
P. arrhenomanes Drechs.	0
P. oligandran Drechs.	0
P. ultimum Trow	0
Phytophthora cinnamomi Rands	1
P. cactorum (Leb. & Cohn) Schroet.	0
P. fragariae Hickm.	0
P. palmivora Butler	0
(Saprolegniales)	
Aphanomyces cochlioides Drechs.	0
A. euteiches Drechs.	0

TABLE I—*Continued*

Fungi tested as hosts	Host reaction[1]
ASCOMYCETES	
Chaetomium globosum Kunze	0
Pezizella lythri (Desm.) Shear & Dodge	0
Sclerotinia sclerotiorum (Lib.) deBary	0
Sordaria fimicola (Rob.) Ces. & de Not.	0
BASIDIOMYCETES	
Coprinus fimetarius Fr.	0
Cyathus sp.	0
Fomes igniarius (L. ex Fr.) Kickx	0
Lentinus lepideus Fr.	0
Omphalia flavida Maubl. & Rangel	0
Polyporus sulphureus Bull. ex Fr.	0
P. versicolor L. ex Fries	0
P. tulipiferae (Schw.) Overh.	0
Schizophyllum commune Fr.	0
FUNGI IMPERFECTI	
Ambylosporium botrytis Fres.	4
Aspergillus niger van Tiegh.	0
A. flavus Link	0
A. fumigatus Fres.	0
A. giganteus Wehmer	0
Penicillium roqueforti Thom	0
P. oxalicum Thom	0
Helminthosporium oryzae Miyake & Hori	0
H. sativum Pamm., King & Bakke	0
Coniothyrium fuckelii Sacc.	0
Fusarium lini Bolley	0[2]
Trichoderma lignorum (Tode) Harz	0[2]

[1] Relative susceptibility of fungi to *Rhizoctonia solani;* 4 = very susceptible, 3 = susceptible, 2 = slightly susceptible, 1 = resistant, 0 = immune.
[2] The hyphae of *F. lini* and *T. lignorum* overgrew and parasitized *R. solani.*

protoplasmic hyphae of the Mucorales. Ayers (**1**) reported that *Dispira cornuta* van Tieghem parasitized only members of the Mucorales and Weindling (**12**) noted that *Pythium* and *Phytophthora* spp. were more susceptible to *Trichoderma lignorum* (Tode) Harz than was *R. solani.* More recently, Drechsler (**5**) described *Trichothecium arrhenopum* Drechs. as a parasite of *Pythium graminicolum* Subr. and *Dactylella spermatophaga* Drechsler (**4**) as a parasite of *P. ultimum* Trow and several other *Pythium* species.

PATHOGENIC VARIATION AND PHYSIOLOGIC SPECIALIZATION

The reaction of 59 potential hosts to three highly virulent isolates of *Rhizoctonia solani* was given in TABLE I. It remained to be determined if other isolates of this heterogenous species attacked fungi and if parasitism was specialized. *Pythium debaryanum* and *Rhizopus* sp. were

selected as hosts because of their known susceptibility (TABLE I) and their strikingly dissimilar host-parasite relations.

In TABLE II the reactions of *P. debaryanum* and *Rhizopus* sp. to 20 isolates of the parasite are summarized. Twenty additional isolates of *R. solani* were avirulent to *Rhizopus* sp. On the basis of differential pathogenicity to *Rhizopus* sp. and *P. debaryanum* distinct pathogenic races are apparent. Race 1 is highly virulent on both hosts, races 2 and 3 are weakly virulent on *Rhizopus* sp. and *P. debaryanum* respec-

TABLE II

SUSCEPTIBILITY OF PYTHIUM DEBARYANUM AND RHIZOPUS SP. TO DIFFERENT ISOLATES OF RHIZOCTONIA SOLANI ON POTATO-DEXTROSE AGAR

Isolate of *R. solani*	Source of isolation	Type of host reaction[1]	
		Pythium	Rhizopus
R-19	Soil	4	4
R-4	*Glycine max* Merr.	3	4
R-1	*Fragaria* sp.	3	4
R-2	*Fragaria* sp.	3	4
R-6	*Medicago sativa* L.	3	3
R-5	*Linum usitatissimum* L.	—	3
R-20	*Fragaria* sp.	2	0
R-21	*Fragaria* sp.	2	0
R-22	*Cucumis melo* L.	1	0
R-18	Soil	0	1
R-24	*G. max*	0	1
R-25	*L. usitatissimum*	0	1
R-26	*M. sativa*	0	2
R-27	*G. max*	0	1
R-28	Unknown	0	0
R-29	*G. max*	0	0
R-30	*Avena sativa* L.	0	0
R-31	*Melilotus alba* Desr.	0	0
R-32	*L. usitatissimum*	0	0
R-33	Unknown	0	0

[1] Relative susceptibility of fungi to *Rhizoctonia solani;* 4 = very susceptible, 3 = susceptible, 2 = slightly susceptible, 1 = resistant, 0 = immune.

tively, and race 4 is avirulent. Thus parasitism is specialized; a given host may be susceptible to one isolate of *R. solani* while immune to another. The data in TABLE II are not intended to show the prevalence of isolates of *R. solani* with the facility to attack fungi. It would not be surprising, however, to find a large number of parasitic races. In a recent review on the parasitism of cultivated plants by *R. solani,* Kernkamp *et al.* (**7**) wrote: ". . . it is obvious that the species *Rhizoctonia solani* comprises an indefinite number of races that differ culturally as well as pathogenically."

There is no apparent relationship between the capacity of *R. solani* to parasitize angiosperms and its capacity to parasitize fungi. According to Gibler (6) isolate R-31 (SCIA of Gibler) was highly virulent and isolate R-30 (252d of Gibler) was weakly virulent on flax, soybean, and pea. However, as shown in TABLE II, neither of these isolates was a parasite of the fungi tested.

OBSERVATIONS ON THE MODE OF PARASITISM

Evidence against an antibiotic. Apparently *Rhizoctonia solani* does not produce an antibiotic or other harmful substances which diffuse through the substrate in advance of the hyphae. Partial proof of this was obtained by the examination of hyphae of *Mucor recurvus* and *Pythium debaryanum* in vertical sections of agar from areas parasitized and overgrown by *R. solani* (isolates R-1 and R-2). The uninfected hyphae were normal in appearance and grew when transferred to fresh media.

Further evidence was obtained by growing isolates R-1 and R-2 of *R. solani,* singly, with *P. debaryanum, P. butleri, Rhizopus* sp. and *M. recurvus* in approximately 20 ml of potato-dextrose broth in Petri dishes. The broth was inoculated by methods previously described. Direct microscopic observations of the host hyphae were made as the colonies grew together. In no instance was there a deleterious effect on the host hyphae in advance of the parasite; nor was there inhibition of the host colonies previous to the meeting with *R. solani.* In addition, the culture liquid obtained from 6- and 48-day-old cultures of *R. solani* grown in rice broth and potato-dextrose broth had no visible effect on spore germination and growth of *M. recurvus.*

Formation of hyphal coils and infection hyphae in the absence of a host. The nature of the stimulus responsible for the formation of penetration hyphae and hyphal coils by *R. solani* is necessary for an understanding of the mechanism of parasitism. The following experiments were made to determine if these structures were formed as the result of a mechanical stimulus or by other means. Glass tubing was drawn out to a diameter of 30 to 45 μ, cut into sections approximately 1 cm in length, and inserted vertically along a line in the center of Petri plates containing potato-dextrose agar. In other plates glass wool, with fibrils about 8 μ in diameter, was placed on the surface of the agar. *R. solani* (isolates R-1 and R-2), alone and with *P. debaryanum,* was inoculated approximately 4 cm from the tubes and glass wool. After the mycelium had grown 1 to 2 cm past the center of the plate, the materials were removed and examined microscopically. *R. solani* responded thigmo-

tropically to both forms of glass but coiling was sparse. On the surface of the larger tubes coils arose mainly as side branches from runner hyphae as in FIG. 2, C; coils formed around the small glass fibrils were tight and arose primarily from a single growing point. The presence of *P. debaryanum* seemed to have no influence on the amount or intensity of coiling by the parasite.

When sterile, acid-washed, cotton fibers (lint hairs) were substituted for glass wool, hyphae of *R. solani* occasionally penetrated the wall of the fibers (FIG. 3, D). The invading hyphae enlarged to fill the lumen but the wall of the fiber was not distended as in the case of a susceptible host (FIG. 3, A). There was no visible destructive effect of the hyphae of *R. solani* on cotton fibers. Fibers in contact with the parasite stained the same blue color with zinc-chloriodide as those not exposed to *R. solani*. It is probable that cellulose hydrolysis did not take place. This probability was strengthened by the failure of *R. solani* to grow in a Czapek's mineral solution with cotton fibers as the only source of carbon. The fungus grew well, however, in the mineral solution plus glucose. Thus it is concluded that, to a limited degree, *R. solani* can coil around and penetrate inanimate filaments comparable in size to host hyphae and that these phenomena occur as a result of a contact stimulus or stimuli.

It can be inferred from the experiments with cotton fibers that penetration of the host hyphae occurs as a result of mechanical pressure. The internal parasitic hyphae of *R. solani* isolates used did not visibly hydrolyze or decompose the walls of *M. recurvus* or other hosts used in these investigations. It would seem likely that if the penetration peg decomposed the cell wall of the host, masses of internal parasitic hyphae in contact with the host wall would produce a similar effect. The probability of cellulose in the hyphal walls of *M. recurvus* further suggests penetration by pressure, since the isolates of *R. solani* involved apparently have no cellulytic enzymes.

THE INFLUENCE OF NUTRITION ON PARASITISM

In early studies it was noted that *Rhizoctonia solani* parasitized *Mucor recurvus* and *Rhizopus nigricans* on PDA but not on water agar (20 g agar/1000 ml H_2O). Thus it seemed that nutrition was a factor in determining the intensity of parasitism. This hypothesis was tested by growing *R. solani* with *M. recurvus* on a number of different substrates given in TABLE III. The grain media and soil were saturated with water; no water was added to carrot, potato and peas. All media were autoclaved at 121° C for 30 minutes. The results are summarized in TABLE III. *R. solani* was highly virulent to *M. recurvus* on corn-

meal, oat grain, and rice grain and avirulent on fresh peas and wheat germ. All media, except muck soil, supported good growth of both host and parasite. The mycelium of *M. recurvus* on wheat germ was strikingly different from that on the other media. The turf was very short (± 2 cm) and sporangiophores were densely protoplasmic; none were attacked by the parasite.

The influence of nutrition on parasitism was further studied by altering only the carbohydrate component of the medium. *M. recurvus* was again employed as a host.

Each carbohydrate tested (TABLE IV) was added, prior to autoclaving, in a 1% concentration to a medium containing the following (in grams per liter of medium): Agar, 15; $NaNO_3$, 3; KH_2PO_4, 1; K_2HPO_4, 1; $MgSO_4 \cdot 12H_2O$, 0.5; and trace elements after Perkins

TABLE III

SUSCEPTIBILITY OF MUCOR RECURVUS TO RHIZOCTONIA SOLANI, ISOLATE R-1, ON DIFFERENT MEDIA

Substrate	Type of host[1] reaction	Substrate	Type of host reaction
Cornmeal (yellow)	4+	Czapeks agar	3
Oat grain	4	Wheat grain	3
Rice grain (polished)	4	Muck soil	2
Potato slices	3	Wheat germ	0
Carrot slices	3	Peas (fresh frozen)	0
Barley grain	3	Water agar	0

[1] Relative susceptibility of fungi to *Rhizoctonia solani;* 4 = very susceptible, 3 = susceptible, 2 = slightly susceptible, 1 = resistant, 0 = immune.

(8). *R. solani* and *M. recurvus* were inoculated simultaneously on each medium by methods previously given and the cultures were incubated at 25° C. The vegetative growth of the host and parasite was given growth ratings based upon the density and radial extension of the mycelium. The results are presented in TABLE IV.

It is interesting to note that *M. recurvus* was susceptible only when the host and parasite were supplied with a hexose or a di-, or polysaccharide composed of hexose residues. When pentose sugars were the source of carbon, infection of the host was sparse or absent. Other results are: (1) growth of the host and parasite was sparse and parasitism absent on media containing the alcohols of glucose, mannose, and galactose; (2) when host and parasite were supplied with galactose or lactose, the latter a disaccharide containing a galactose residue, infection was very sparse; (3) although cellobiose and maltose differ only by the nature of the linkage separating the two glucose residues, they differ

markedly in their effects on growth and parasitism. On maltose, *M. recurvus* was susceptible to *R. solani;* on cellobiose it was resistant.

It is evident from the results given in TABLES III and IV that the kind of nutrients on which *R. solani* and *M. recurvus* were grown immediately prior to contact of their mycelia greatly influenced parasitism. Although complex media such as cornmeal and rice grain altered parasitism, *R. solani* developed maximum parasitic activity on a simple medium composed of inorganic salts and a single carbon source.

TABLE IV

THE INFLUENCE OF CARBOHYDRATES ON SUSCEPTIBILITY OF MUCOR RECURVUS TO RHIZOCTONIA SOLANI, ISOLATE R-1

Carbohydrate	Type of host reaction[1]	*R. solani*	*M. recurvus*
		Veg. growth[2]	Veg. growth[2]
l (+) rhamnose	0	2	3
l (−) rhamnose	0	1	1
l (+) arabinose	0	1	9
d (+) xylose	1	10	8
d (+) galactose	1	12	10
ducitol	0	2	2
d (−) levulose	4	12	10
d (+) mannose	3	10	10
d (−) mannitol	0	2	2
d-sorbitol	0	3	4
d-glucose	3	10	10
d (+) lactose	0	5	2
d (+) maltose	3	12	10
sucrose	3	10	10
cellobiose	1	9	10
d (+) raffinose	1	12	1
Starch	3	10	10
Glycogen	1	9	9
Inulin	0	2	4

[1] Relative susceptibility of fungi to *Rhizoctonia solani;* 4 = very susceptible, 3 = susceptible, 2 = slightly susceptible, 1 = resistant, 0 = immune.

[2] Numbers, graduated in units from 1 to 12, represent relative amounts of mycelial growth. 1—very sparse, mycelial development barely visible; 12—profuse growth.

Similar qualitative results were obtained by Ayers (1) for *Dispira cornuta* Van Tiegh. On the other hand Weindling (12) concluded that the parasitism of *Trichoderma lignorum* occurred independently of any medium whenever *T. lignorum* was actively growing and had sufficient moisture.

EFFECT OF ENVIRONMENT ON PARASITISM

Temperature. A detailed study on the relation of temperature to infection is beyond the scope of the present paper. Neverthless, it

seemed desirable to determine the potential importance of temperature in altering host-parasite relations. This was accomplished by growing *Mucor recurvus* with the parasite at 15, 20, 25 and 30° C.

M. recurvus was susceptible at 25 and 30° C, slightly susceptible at 20° C and immune at 15° C. While *Rhizoctonia solani* appeared to grow well at each of the preceding temperatures, *M. recurvus* was strongly inhibited at 15° C. The protoplasm of the sporangiophores was very dense and dark brown in color. A few sporangiophores of this type were observed at 20° C; they were never infected by *R. solani*. Immune sporangiophores of a similar type were previously observed on autoclaved wheat germ.

TABLE V

SUSCEPTIBILITY OF FUNGI TO RHIZOCTONIA SOLANI ON POTATO-DEXTROSE AGAR IN LIGHT AND DARK

Fungus	Type of host reaction[1]	
	Light	Dark
Rhizopus sp.	0	4
Mucor recurvus	1	3
Pythium debaryanum	1	3
Pythium butleri	0	2

[1] Relative susceptibility of fungi to *Rhizoctonia solani*; 4 = very susceptible, 3 = susceptible, 2 = slightly susceptible, 1 = resistant, 0 = immune.

Thus, temperature had a marked influence on parasitism. The host reaction of *M. recurvus* was changed from susceptible at 25° C to immune at 15° C and growth of both organisms occurred over a wider range than did parasitism.

Light. Diffuse daylight normally present in the laboratory had no observable influence on the infection of hosts by *R. solani* but artificial light of a higher intensity seemed to suppress parasitism. To determine the effect of light *R. solani* was grown with each of the fungi listed in TABLE V at 25° C in continuous white fluorescent light. The flasks were placed 40 cm from the light source; intensity at this distance ranged from 150 to 400 ft candles. Control flasks were incubated in darkness at the same temperature. The results are given in TABLE V.

Vegetative growth of each of the fungi was suppressed in the light. The colonies of *M. recurvus* and *Rhizopus* sp. were approximately 2.5 cm in height in the light and 8–10 cm in darkness. The inhibition of infection by bright light is correlated with the inhibition of growth of both host and parasite. As noted previously, conditions responsible for poor vegetative growth of either *R. solani* or a host fungus caused a

reduction in the amount of infection. This observation is supported by the experiments with light.

The influence of other fungi. In the course of experimental work, contaminating fungi occurred from time to time in flasks containing the parasite and a susceptible host. On all media, the presence of most foreign fungi except Phycomycetes appeared to inhibit infection by *R. solani.* This observation was substantiated by inoculating each of several fungi between the advancing colonies of *M. recurvus* and *R. solani,* isolate R-1; the same fungi also were inoculated alone with *M. recurvus* and *R. solani* respectively. The medium employed was the basal medium used for carbohydrate studies with the addition of 1% glucose. Colonies of the host and parasite were two days old at the time spores of the third fungus were introduced.

Aspergillus fumigatus completely inhibited the infection of *M. recurvus* by *R. solani.* In the presence of *Aspergillus flavus, Penicillium oxalicum, P. roqueforti* and *Chaetomium globosum, M. recurvus* was only slightly infected (Reaction Type 1). *Helminthosporium oryzae* and *Spondylocladium* sp. had no significant effect on infection and *Trichoderma lignorum* and *Fusarium lini* parasitized both *M. recurvus* and *R. solani.* In the latter relationships it was impossible to measure the effects of *R. solani* on *M. recurvus.*

Thus certain fungi grown singly on the same substrate simultaneously with *R. solani* and *M. recurvus* either completely inhibited, limited or had no effect on the parasitic capabilities of *R. solani.*

DISCUSSION

The evidence presented here demonstrates that *Rhizoctonia solani* actually parasitizes other fungi. This conclusion is based upon the abundance of parasitic coils observed around the hyphae of susceptible fungi, disorganization of host protoplasm, and the extensive growth of *R. solani* inside host thalli.

The destructive action of *R. solani* is apparently quite different from that reported by Weindling (**12**) for *Trichoderma lignorum.* The latter organism produces an antibiotic which diffuses in advance of its hyphae and kills other fungi. Unlike *T. lignorum, R. solani* requires an intimate relationship with other fungi and has no observable deleterious effects at a distance.

Although the chemical basis of parasitism remains unknown, a consideration of the mode of infection and the behavior of different strains of *R. solani* provides some understanding of the nature of parasitism. The mode of attack is not a chance relationship in which the parasite

forms coils at one time and infection hyphae at another; rather it is a specific reaction. The following relationships illustrate this point: isolate R-1 of *R. solani* attacks *Pythium debaryanum* almost entirely by the formation of tight coils around its hyphae, while it parasitizes *Syncephalastrum* sp. exclusively by penetrating its hyphae and sporophores and forming an internal parasitic mycelium. Furthermore, the manner in which *R. solani* attacks other fungi apparently depends upon both *R. solani* and the fungus host. This point is illustrated by the following: isolate R-1 of *R. solani* coils around and penetrates the hyphae of *Rhizopus* sp. but only coils around the hyphae of *P. debaryanum;* isolate R-26 only penetrates *Rhizopus* sp.; it neither coils around nor penetrates the hyphae of *P. debaryanum.* In the relationships of isolate R-1 of *R. solani* with *P. debaryanum* and *Rhizopus* sp., respectively, the host apparently controls the mode of attack, for although isolate R-1 has the capacity to form penetration hyphae it attacks *P. debaryanum* solely by the formation of hyphal coils. Conversely, in the relationships of isolate R-26 of *R. solani* with *P. debaryanum* and *Rhizopus* sp., respectively, the mode of attack is apparently determined by the parasite, since isolate R-26 penetrates *Rhizopus* sp. but does not form hyphal coils around the hyphae of either *Rhizopus* sp. or *P. debaryanum.* Isolate R-26 apparently lacks the capacity to form parasitic coils. The hyphae of *R. solani,* alone, will coil weakly around glass rods, comparable in size to the hyphae of host fungi, and occasionally penetrate the walls of cotton fibers. A suitable host fungus is required, however, for abundant formation of these parasitic structures.

Thus it appears to the writer that hyphal coils and infection hyphae are formed by *R. solani* as a result of chemical stimuli produced on the surface of the hyphae of the host and parasite respectively. These substances seem to act in a complementary manner to determine the mode of infection. It could be further postulated that the relative amounts of each of the substances produced by host and parasite determine the intensity of penetration and formation of parasitic coils.

Parasitism depends upon a balance of nutritional and environmental factors: temperature, light and composition of the substrate, each exert an effect. Good vegetative growth of both the host and parasite appears to be requisite for infection. If, for example, *M. recurvus* and *R. solani* are grown together on water agar where growth of both fungi is scant, infection does not occur. Likewise, if the same fungi are grown on a medium containing only rhamnose or arabinose as a source of carbon, no infection results. The former carbohydrate supports good growth of the parasite only; the latter of the host only. However, good vegeta-

tive growth, *per se*, does not insure that parasitism will occur. *R. solani* and *M. recurvus* both grew luxuriantly on autoclaved peas (TABLE III) but infection was absent.

During the last decade the trend of research on the antagonistic associations among soil organisms has been largely concerned with antibiosis, *i.e.*, with the harmful effects of one organism upon another by the liberation of chemical substances. Many antifungal and antibacterial substances have been isolated from Actinomycetes and bacteria and chemically identified. Various workers have pointed out the possibilities of controlling soil-borne plant diseases by the addition of antibiotic microorganisms. Little attention has been given to parasitism among soil fungi. The writer is aware of only two reports (**2**, **11**) in which parasitism was demonstrated to occur in the soil. However, considering the profound selective influence of parasitism in other plant and animal communities, it seems reasonable to assume that parasitism plays a similar role among soil organisms.

R. solani is not the only plant pathogen with the capacity to parasitize fungi. In the present study isolates of *Fusarium lini*, a parasite of flax, attacked the hyphae of *R. solani* in much the same way that *R. solani* parasitized other fungi. It would seem that certain plant pathogens may have two or more systems for parasitism; possibly these capacities are related to survival of these organisms. It is well known, *e.g.*, that *F. lini* persists in soil for long periods of time in competition with other organisms.

SUMMARY

Rhizoctonia solani Kühn, an important soil-inhabiting parasite of cultivated plants, also parasitizes fungi. Except for *Ambylosporium botrytis* of the Fungi Imperfecti, only species of Phycomycetes were parasitized. *R. solani* attacks fungi by penetrating their aerial hyphae and establishing an internal parasitic mycelium or by coiling around their aerial hyphae or by a combination of both. The host-parasite relations are described.

Evidence is presented that *R. solani* does not secrete an antibiotic effective in killing fungi at a distance. The hypothesis is presented that the host fungus and *R. solani* produce complementary chemical stimuli responsible for the development of parasitic hyphal coils and infection hyphae.

Isolates of *R. solani* differ in virulence from highly virulent to avirulent. Four physiologic races were recognized on the basis of differential pathogenicity to *Pythium debaryanum* and *Rhizopus* sp.

The effects of nutrition, temperature, light and other fungi on parasitism are given. The results of these studies indicate that good growth of the host and parasite was prerequisite for parasitism. Two isolates of *R. solani* produced basidiospores in culture. This is believed to be the first report of a basidiomycete parasitizing fungi belonging to the Phycomycetes and Fungi Imperfecti.

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MODE OF PARASITISM AND HOST RANGE OF PIPTOCEPHALIS VIRGINIANA [1]

CHARLES R. BERRY AND H. L. BARNETT

(WITH 12 FIGURES)

The genus *Piptocephalis* belongs to the order Mucorales. The characteristic slender, dichotomously branched sporophores, which terminate in deciduous head cells bearing clusters of spore-chains, make this genus easily distinguishable from all others of this order. All known species are obligate parasites of other fungi.

The contributions of most of the early workers to our knowledge of *Piptocephalis* were mainly taxonomic (de Bary, **1865**; van Tieghem, **1875**; Bainier, **1883**; Vuillemin, **1887**). Brefeld (**1872**) studied the morphology, mode of parasitism and zygospore formation of *P. freseniana*. Matruchot (**1900, 1903**), using a species which he described and named *P. tieghemiana*, was the first to make an extensive study of the host range. He tested representatives of all classes of fungi, and found parasitism to occur on ten members of the Mucoraceae, and one of the Pilobolaceae. No species tested outside the order Mucorales was parasitized.

Until recently, no species of *Piptocephalis* had been found to parasitize any fungus outside the Mucorales. Dobbs and English (**1954**) described and named a new species, *P. xenophila*, which not only parasitizes certain species of Ascomycetes and Fungi Imperfecti, but grows and sporulates better on these hosts than on some species of Mucorales.

The work of Ayres (**1933, 1935**), on the parasitism of *Dispira cornuta*, is of interest because of the similarity of this fungus in morphology, mode of parasitism and host range to most species of *Piptocephalis*. However, *D. cornuta* is able to grow on certain high nitrogen media in the absence of a host.

Piptocephalis virginiana (Leadbeater, **1957**) can be grown easily on host fungi cultured on a variety of synthetic media. Since only a minimum of time and equipment are needed, it offers an excellent opportunity to study the obligate, or balanced, type of parasitism, particularly

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the effects of nutrition and environment on the growth and development of the parasite. The present investigation is an attempt to learn more about the basic principles of parasitism by studying the amount of growth and abundance of sporulation of *P. virginiana* when the host fungi were cultured under different conditions. This paper reports the results of a part of this study, including the morphology of the parasite, spore germination, mode of parasitism, growth of the parasite and host range.

MATERIALS AND METHODS

The isolate of *Piptocephalis virginiana* used in this study appeared on *Mucor hiemalis* which was collected in a cave near Morgantown, West Virginia, in 1953 by Foster L. Brown, a former graduate assistant in this department. Illustrations and a brief description of this isolate are presented later in this paper.

The obligate nature of this parasite necessitated its culture on a susceptible host. Collecting the spores of the parasite free from the spores of the host at first posed a difficult problem. *Mucor ramannianus* was the first host selected for this purpose because of its low habit of growth. The sporophores of the parasite grew well above the host and spores could be picked off easily by touching them with a bit of agar on a needle. This method was discarded because a high percentage of collections were contaminated with host spores and because of the demand for greater amounts of inoculum.

Choanephora cucurbitarum was then selected as the standard host for the production of spores of the parasite. It has been shown that the asexual sporulation of this host fungus is reduced to a minimum by allowing it to grow either in continuous bright light, or darkness, and by allowing the carbon dioxide to accumulate in the culture vessel (Barnett and Lilly **1950, 1955**). *C. cucurbitarum* and the parasite were grown together on a malt extract-yeast extract agar (20 gm malt ext., 2 gm yeast ext., 20 gm agar, 1 liter water, pH 6) in 250 ml Erlenmeyer flasks in continuous darkness.

A heavy crop of *P. virginiana* spores could be harvested from the aerial cover of sporophores after five days. The spores of *P. virginiana* were dry and easily dislodged. They were collected by means of air suction into sterile test tubes. The spores were then placed in distilled water and stored in the refrigerator. Samples from each tube were plated on agar to determine if *C. cucurbitarum* spores were also present, and contaminated tubes were discarded. When kept at about 5° C in distilled water the spores remained viable over a period of six months

and served as a ready and convenient source of uniform inoculum. When frozen in distilled water, spores remained viable for twenty months.

The medium used in the host range experiments was composed of 3 gm of malt extract, 1 gm of yeast extract, 20 gm of agar and 1 liter of water. The pH was adjusted to 6.0 before autoclaving. This medium was selected because it supported good growth of all the fungi tested and allowed germination of *P. virginiana* spores. A modification of the liquid, glucose-asparagine medium suggested by Lilly and Barnett (**1951**, p. 427) was used for culture filtrate studies and some other experiments. The modified medium contained only 3 grams of glucose to the liter. Various solutions were used in spore germination experiments. The temperature of incubation was approximately 25° C.

The production of branched sporophores of *P. virginiana* bearing clusters of spore chains was used as the criterion of parasitism. In no case was the parasite observed to become successfully established on a host without sporulating. When the sporophores were abundant they could be recognized easily with the unaided eye, but when sporulation was light it was frequently necessary to use the stereoscopic microscope to determine the presence of the parasite.

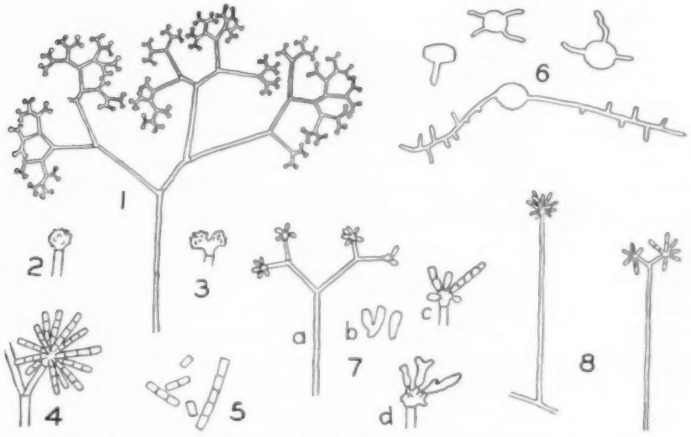
A number of host fungi were selected from the West Virginia University culture collection. Others were kindly furnished by Dr. C. W. Hesseltine, Dr. J. W. Carmichael, Dr. M. P. Backus and Dr. L. Shanor. A culture of *Piptocephalis xenophila,* furnished by Dr. C. G. Dobbs, was compared with our isolate in some spore germination studies.

MORPHOLOGY OF THE PARASITE

The mycelium and asexual fruiting structures of *Piptocephalis virginiana* resemble those of *P. freseniana* as described by Brefeld (**1872**). The general appearance of the sporophores (FIG. 1), the shape and size of the head cell and spores (FIGS. 2–5), and the germinating spores (FIG. 6) strongly resemble Brefeld's drawings. However, the zygospores of this species (FIG. 12), which were discovered only recently in this laboratory (unpublished), are distinctly different from those of *P. freseniana.*

In mass, the sporophores are tan against the background of the host mycelium. The sporophores are usually 5 mm or more in length. They are upright at first and later bend over as if by the weight of the developing fertile, branched portion. The main stem of the sporophore is approximately 7 μ in diameter, with its branches somewhat smaller.

The sporophore is septate and has fine longitudinal striations. No rhizoids or stolons are present, although new mycelium may be produced where tips of young sporophores bend over far enough to touch the agar. The upper portion of the sporophore is dichotomously branched 4 to 8 times. The ultimate branches terminate in enlarged globose to bluntly obconic head cells, each bearing up to 25 sporing-rods. These sporing-rods are straight or slightly curved, approximately 18 to 25 μ in length and break up at maturity into 4 to 7 cylindrical rod-like spores which average approximately 5.3 × 2.6 μ. The head cells are 5 to 9 μ wide



FIGS. 1–8. *Piptocephalis virginiana*. 1. Dichotomously branched sporophore, × 100. 2, 3. Head cells, × 500. 4. Spore chains attached to head cell, × 400. 5. Spores, × 425. 6. Germinating spores, × 800. 7. Anomalies. a. sporophore, × 200; b. spores, × 400; c, d. spores and head cells, × 400. 8. Dwarf sporophores, × 200.

and are deciduous. They are tuberculate in appearance after shedding the spores and sometimes are cleft or lobed at the apex (FIGS. 2, 3). As in most other Mucorales, sporulation is rapid, the whole process requiring no longer than 24 hours.

The morphology of the parasite and the production of spores have been remarkably uniform when the host is grown on a malt extract-yeast extract medium. However, in the course of culturing host fungi on a variety of semisynthetic media (to be discussed in a later paper) several anomalies in morphology have been observed. A few of these are illustrated in Fig. 7.

EXPERIMENTAL RESULTS

Spore Germination. In preliminary experiments it was noted that the spores of *Piptocephalis virginiana* failed to germinate in distilled water or on water agar. On malt extract-yeast extract agar in the absence of a host fungus the spores swelled, becoming nearly globose, about 10 to 12 μ in diameter, and the first germ tube was produced within 18 hours. The percentage of germination increased until it reached 90 percent or greater, with some germ tubes as long as 40 μ within 24 hours. Each spore produced from one to five slender germ tubes which averaged about 1.5 to 2.5 μ in diameter. The germ tubes produced numerous short, right angle branches and continued to grow for a short time. It appeared that the growing hyphae and germ tubes were non-septate, but septa were produced later at branches or segments devoid of protoplasm.

A number of substances were tested to determine the occurrence of the factor or factors needed for germination. The materials were autoclaved in distilled water and drops of the solution were placed on glass slides with spores of the parasite. The percentage of germination was 90 percent or higher in yeast extract, nutrient broth, neopeptone, rabbit dung decoction, and enzymaticly hydrolyzed casein (Nutritional Biochemical Corp.). An extract of *P. virginiana* spores also allowed high germination. Soy protein (Nutritional Biochemical Corp.) and fresh orange juice each allowed about 40 percent germination. Germination in malt extract and an extract of whole wheat flour was less than 10 percent.

Synthetic media containing L-glutamic acid, a carbon source, such as glucose, mannose or fructose, and micro elements (Fe, Zn, Mn, Ca) permitted about 25 percent germination after 48 hours at 25° C. The removal of glutamic acid, carbon source, or micro elements from the medium resulted in an almost complete loss of its ability to support germination. Ten other amino acids were tested but none could be substituted for glutamic acid. Agar and liquid media permitted approximately the same amount of germination.

Compounds which did not support germination were: water agar, water, glucose, 16 amino acids used singly, acid hydrolyzed casein (Nutritional Biochemical Corp.), a mixture of B vitamins (thiamine, biotin, inositol, pyridoxine), vitamin B_{12}, coprogen, nucleic acid, sodium nucleinate, guanine, uracil, glutathione, glycylglycine and several synthetic media including glucose-asparagine.

Germination occurred on glucose-asparagine agar only in the presence of certain other fungi. This suggested that a spore germination

factor was produced and excreted by other fungi. Filtrates of 3- or 4-day old cultures of several fungi growing in liquid glucose-asparagine media were then tested for the germination factor after they were sterilized by filtration or by autoclaving. Filtrates of *Choanephora cucurbitarum* and *Thamnidium elegans* supported 50 to 90 percent germination within 24 hours. The filtrates from 7-day old cultures of these two fungi were much poorer than those from 3-day-old cultures. Less than 50 percent germination occurred in filtrates of *Mortierella pusilla, M. jenkini, Fusarium oxysporum* f. *medicaginis, Rhodotorula* sp., *Penicillium spiculisporum, Trichoderma viride, Botrytis cinerea, Monascus purpureus, Aspergillus rugulosus* and *A. niger.* It should be noted that the spore germination factor is produced by a number of fungi which are not hosts of *P. virginiana.* It is apparently a normal metabolic product of these fungi, since it disappears with time from the culture filtrates. Sterilization of the filtrates by autoclaving (15 lbs for 15 min) did not destroy the germination factor; in fact it seemed to enhance the activity of some of the filtrates. Spores did not germinate in culture filtrates of *Endoconidiophora fagacearum, Penicillium expansum, Glomerella cingulata* or *Helminthosporium sativum.* This failure may have been due to the presence of an inhibitor rather than the absence of a spore germination factor.

There is some evidence that at least two factors are involved in the germination of the parasite spores. When spores are placed in orange juice or a filtrate of *Choanephora cucurbitarum,* germination is quick with little swelling. In some solutions, such as enzymaticly hydrolyzed casein and beef extract, the spores may swell and become globose but few if any germinate. Swelling does not occur in distilled water. It seems likely that one factor governs swelling of spores while another is necessary for the production of germ tubes.

Brefeld (**1872**) did not mention a germination factor for *P. freseniana,* but if this species requires a spore germination factor it may have been overlooked, since he used dung extract as a culture medium for much of his work.

Dobbs and English (**1954**) have reported that spores of *P. xenophila* do not germinate in the absence of a host fungus. In our limited trials with the same isolate of *P. xenophila,* the spores germinated within 48 hours in the absence of a host on either a malt extract-yeast extract agar or a horse dung extract agar. These differences were probably due to the use of different media in the two laboratories. Germination was normal for the fungus and the germ tubes reached a length of at least 35 μ before growth ceased. Not enough trials have been made to de-

termine whether the factors for germination are the same in the two species.

Limited Development in the Absence of a Host. On a malt extract-yeast extract medium the germ tubes of *P. virginiana* produced several branches and the hyphae reached a length of 75 to 100 μ before they ceased to grow. Some of these young mycelia (estimated as approximately 10 percent) were vigorous enough to produce an upright dwarf sporophore which terminated in one or two heads of spores (FIG. 8). Only occasionally was the sporophore forked, and usually it was abortive and seldom exceeded 300 μ in length. The spore chains were few, short, and frequently composed of only two or sometimes four spores. Abnormalities were frequent. These spores were collected and placed on other plates of the same medium. Less than half of them germinated and in most cases the germ tubes remained short and unbranched. This suggests that the spores produced by the parasite growing on a host contain certain stored materials which are beneficial to further development. In the absence of a host this material is used or becomes diluted so as to become ineffective. Evidently this substance is different from the spore germination factor discussed above.

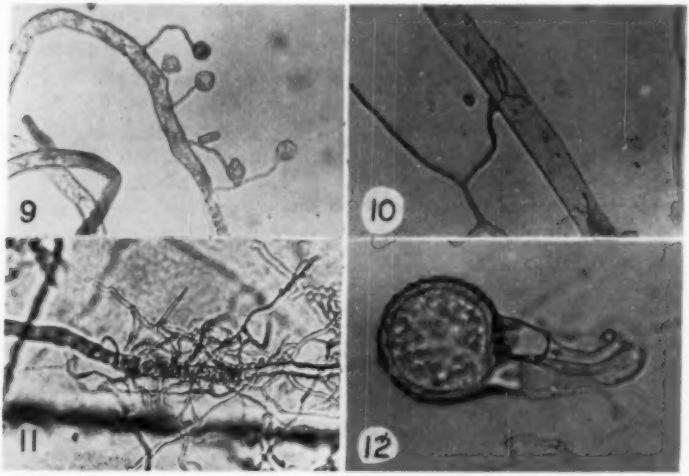
All attempts to induce further development of the parasite mycelium in the absence of a host fungus have failed. The various media tested included dung agar, killed mycelium of susceptible hosts, filtrates of host fungi, and cooked and raw egg yolk. The last two materials were found by Ayers (**1933**) to support the growth of *Dispira cornuta*.

Mode of Parasitism. All known species of *Piptocephalis* are obligate parasites which penetrate the hyphae of the host fungi and produce branched haustoria (van Tieghem, **1875**; Bainier, **1906**; Brefeld, **1872**; Matruchot, **1903**; Dobbs and English, **1945**). This is also true of *P. virginiana* (FIG. 10).

When spores of this species germinated near the hyphae of a susceptible host, the germ tubes frequently showed a definite attraction toward the host. This was especially true when *Thamnidium elegans* and *Choanephora cucurbitarum* were the hosts. The parasite spores usually put out a single germ tube from the side nearest the host. The germ tube then grew directly toward the host, making contact and penetrating the hypha before forming any branches (FIG. 9). The production of a small appressorium-like swelling by the parasite where the germ tube makes contact with a susceptible host is followed by penetration by a fine hyphal peg. The formation of branched haustoria occurs after penetration (FIG. 10). Germination, penetration and formation of haustoria required as little as 24 hours, when parasite

and host were in close proximity. Branches of the parasite mycelium were soon formed after the initial penetration and penetrated host hyphae at numerous points (FIG. 11).

The first sporophores of *P. virginiana* appeared on cultures about three days old. Subsequent development on highly susceptible hosts was rapid and in Petri dish cultures the maximum growth of the parasite occurred within five days. On more resistant hosts, growth of the parasite was slower. The spread of the parasite over the host culture appeared to be accomplished by multiple penetration of the host.



FIGS. 9–12. *Piptocephalis virginiana.* 9. Germinating spores growing towards host hypha, × 475. 10. Penetration of host hypha showing appressorium-like swelling and haustoria, × 650. 11. Parasite mycelium surrounding hypha of *Mycotypha microspora,* × 400. 12. Zygospore, × 475.

In most cases, *P. virginiana* caused no apparent damage to the host fungi. However, it was observed that sometimes the parasite retarded the growth of *Mycotypha microspora.* When grown on glucose-glutamic acid media (glucose 3.0 gm, glutamic acid 2.0 gm, KH_2PO_4 1.0 gm, $MgSO_4$ 0.5 gm, micro elements 2.0 ml and thiamine 100 μgm), the dry weight of the mycelia of the host-parasite combination was less than 50 percent of the dry weight of host mycelium grown alone.

When spores of *P. virginiana* were sown with an immune fungus which produced the spore germination factor, the germ tubes were neither attracted to, nor did they penetrate the mycelium. Immunity,

in this case, may have been due to failure of the parasite to penetrate the host.

Host Range. Few extensive studies of the host range of species of *Piptocephalis* have been made, but it has been generally accepted that their host species are confined to the order Mucorales. This was the conclusion of Matruchot (**1903**), who tested a variety of fungi as possible hosts of *P. tieghemiana.* It is of some interest that Ayres (**1935**) obtained similar results with *Dispira cornuta,* a closely related fungus. However, Dobbs and English (**1954**) found that *P. xenophila* parasitized several species of Ascomycetes and imperfects and grew as well or better on these as on species of Mucorales.

Piptocephalis virginiana has been found in the present study to parasitize only certain species of Mucorales. None of the twenty species tested outside this order supported growth of the parasite.

The species of Mucorales found in this study to be susceptible are given in the following list:

Mucoraceae
- *Absidia cylindrospora*
- *A. orchidis*
- *Circinella minor*
- *Mucor genevensis*
- *M. hiemalis*
- *M. ramannianus*
- *M. spinescens*
- *M. varians*
- *Phycomyces blakesleeanus* (−, +)
- *Rhizopus nigricans*
- *Sporodinia grandis*
- *Zygorhynchus vuillemini*

Thamnidiaceae
- *Cokeromyces recurvatus*
- *Helicostylum* sp.
- *Thamnidium elegans*

Piptocephalidaceae
- *Syncephalastrum racemosum*

Cunninghamellaceae
- *Cunninghamella elegans*
- *C. echinulata*
- *Mycotypha microspora*

Choanephoraceae
- *Choanephora cucurbitarum* (+, −)
- *C. (Blakeslea) trispora*

Mortierellaceae
- *Mortierella pusilla*

Not all of the host fungi supported equal growth of the parasite under the same conditions. The most rapid and abundant growth of *P. virginiana* was made on *Absidia orchidis, Thamnidium elegans* (at 25° C), *Syncephalastrum racemosum, Cunninghamella elegans, Mycotypha microspora* and *Mortierella pusilla.* Growth of the parasite was generally poor on *Phycomyces blakesleeanus* and *Rhizopus nigricans.*

The following species were tested repeatedly and did not support growth of *P. virginiana*:

Coemansia pectinata
Coemansia sp.
Endogone malleola
Mortierella bainieri
M. candelabrum
M. jenkini
M. marburgensis
M. minutissima
M. polycephala
M. simplex
M. tuberosa
Pilobolus sp.

Twenty species outside the Mucorales which were tested but not parasitized by *P. virginiana* are listed below:

Achlya sp.
Aspergillus clavatus
A. niger
A. rugulous
Cladosporium sp.
Collybia velutipes
Guepinia spathularia
Itersonilia sp.
Monascus purpureus
Penicillium chrysogenum
P. expansum
P. spiculisporum
Peronospora parasitica (on *Dentaria*)
Phytophthora infestans
Phytophthora sp.
Phythium sp.
Polyporus cinnabarinus
Rhodotorula sp.
Saccharomyces cerevisiae
Tridentaria implicans

The host range of *P. virginiana* is similar to that of *P. tieghemiana* (Matruchot, **1903**), but is quite unlike that of *P. xenophila* (Dobbs and English, **1954**).

DISCUSSION

The parasitic relationship between species of *Piptocephalis* and their fungus hosts offers an excellent opportunity to study the various stages in the obligate type of parasitism under conditions controlled to a greater degree than can be achieved with similar relationships involving higher plants. The effects of a number of factors can be tested at various stages from spore germination to sporulation of the parasite, with a minimum of equipment and time.

Piptocephalis virginiana closely resembles other species of *Piptocephalis* in so far as mode of parasitism and host range are concerned. No species of this genus has been grown successfully in the absence of a living host. Parasitism of hosts outside the Mucorales is unusual and has been described only for *P. xenophila* (Dobbs and English, **1954**). That species not only attacks fungi outside but seems to be more at home on some Ascomycetes, especially the Aspergillaceae, than on members of the Mucorales. It is also of interest that Ayers (**1935**) found that the host range of *Dispira cornuta* is limited to the Mucorales and that

the mode of parasitism is similar to that of *Piptocephalis*. Ayers, however, obtained saprophytic growth of *D. cornuta* on certain natural media which were rich in proteins.

The requirement of a spore germination factor by *P. xenophila* and our species, appears to be an adaptation for survival for these parasites. However, compounds other than secretions of host fungi have been found to induce germination under laboratory conditions and it is likely that some spores germinate in nature on various substrata which do not support continued saprophytic growth. The failure of Brefeld, Matruchot and others to note the need for such a germination factor for their species may have been due to their use of natural media containing this factor.

The positive tropism of germ tubes of the parasite to near-by host hyphae also suggests an adaptation for survival. The production of a small appressorium-like swelling by the parasite where the germ tube makes contact with a susceptible host is followed by penetration by a fine hyphal peg. The formation of haustoria after penetration follows the usual pattern for obligate parasites. It is assumed that a host furnishes the parasite with one or more nutrients which are not present in the medium and which the parasite cannot synthesize from the nutrients in the medium. The essential nutrient, if such exists, may be present only in minute quantities, or it may be highly unstable, for in no case did killed host mycelium support growth of the parasite. It is also possible that the parasite may be lacking in one or more enzyme systems essential for converting compounds in the medium into usable nutrients. The enzyme system of the host may then function for itself and the parasite.

Growth of the parasite is dependent upon (1) penetration of the host hyphae, (2) establishment of a compatible nutritional relationship with the host and (3) the absence of toxic substances such as antibiotics in the host mycelium.

Only when the parasite was cultured on *Mycotypha microspora* was there any apparent harmful effect on the host. However, when *M. microspora* was grown with the parasite on glucose-glutamic acid media, there was a definite retardation of growth of the host.

Piptocephalis virginiana was never observed to penetrate the mycelium of a fungus which did not support the growth of the parasite. Therefore, immunity to this parasite may be primarily due to its failure to penetrate the mycelium wall. This hypothesis at present is purely speculative, since we have no information regarding actual differences in cell wall composition or structure of immune and susceptible species

of Mucorales. The age or composition of the cell wall may also be a contributing factor to resistance or susceptibility of a given species under different nutritional conditions, since there is some evidence that age of mycelium is a factor in the development of the parasite.

The host range of *P. virginiana* is of interest. Species from six families and fifteen genera of Mucorales are demonstrated hosts. Members of the Mucorales tested and not parasitized were *Pilobolus* sp., *Coemansia pectinata, Coemansia* sp., *Endogone malleola* and eight species of *Mortierella.* Of this genus only *M. pusilla* was parasitized and it was highly susceptible. It is of interest that only two of the six species of *Mortierella* tested by Ayres were susceptible to *Dispira cornuta.* Previous workers have not reported any species of *Mortierella* (except *M. ramanniana* by Dobbs and English (**1954**), considered here as *Mucor rammanianus*) to be parasitized by any species of *Piptocephalis.* Throughout the present study a striking similarity was observed between the responses of *P. virginiana* on *Mucor ramannianus* and on *Mortierella pusilla.* The morphological resemblance between these two fungi has been pointed out by Christenberry (**1940**). The physiological similarity between these two species indicates that they may belong to the same genus.

SUMMARY

The morphology of *Piptocephalis virginiana* is described briefly. The asexual fruiting structures are similar to those of certain other species of *Piptocephalis.* This species, however, can be distinguished from all other known species on the basis of zygospores alone.

During germination, the rod shaped asexual spores swelled to two or three times their normal size and became globose, then produced from one to several germ tubes. The spores did not germinate in distilled water or on water agar, but required the addition of a nutritional or stimulatory factor. This substance was found in several products such as yeast extract, orange juice, horse dung extract and enzymatically hydrolyzed casein. The only synthetic medium which permitted germination contained glutamic acid, although glutamic acid alone supported little or no germination. On a suitable medium in the absence of a host the spores germinated and a number produced dwarf abortive sporophores. These were generally unbranched and bore only a few spores. No further development followed.

Germ tubes of the parasite are attracted to a near-by host hypha which is penetrated by means of a hyphal peg. Haustoria are then formed inside the host mycelium.

The known host range included fifteen genera belonging to six families of the order Mucorales. The following species of Mucorales did not support the parasite: *Pilobolus* sp., two species of *Coemansia*, *Endogone malleola* and eight species of *Mortierella*. Of the genus *Mortierella* nine species were tested and only *M. pusilla* was found to support the parasite. Twenty species outside the Mucorales were tested but none supported the parasite.

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A NEMATODE-CAPTURING PHYCOMYCETE FORMING CHLAMYDOSPORES TERMINALLY ON LATERAL BRANCHES

CHARLES DRECHSLER [1]

(WITH 1 FIGURE)

The genus *Cystopage* was erected in the Zoopagaceae for fungi that subsist by capturing microscopic animals through adhesion to aseptate filamentous hyphae and that reproduce by forming chlamydospores on or in the substratum (**1**). Three species that I assigned to the genus under the binomials *C. subtilis* (**1**), *C. ellipsospora* (**3**) and *C. sphaerospora* (**3**) are habitually destructive to terricolous rhizopods; while the 2 more robust congeneric forms which I presented under the names *C. lateralis* (**1**) and *C. intercalaris* (**2**), signalizing the positional relations of their chlamydospores, always prey on free-living nematodes. Another species predacious on eelworms is here described as a sixth member of the genus, under an epithet which, being compounded of 2 words (κλαδος, σπορα) meaning "branch" and "seed," respectively, is similarly intended to indicate the usual position of the reproductive bodies.

Cystopage cladospora sp. nov.

Mycelium parce expansum; hyphae incoloratae, continuae, parce ramosae, plerumque 3–6 μ latae, vermiculos nematoideos glutino primum incolorato mox flavo tenentes, integumentum cujusque animalis capti perforantes, ramulos assumentes vulgo 2–3 μ crassos intus evolventes qui carnem exhauriunt; chlamydosporae plerumque sufflavae, globosae vel ellipsoideae, vulgo 20–30 μ in diametro, protoplasmatis valde granulosi repletae, muro primum levi postremo saepe aliquid undulato circumdatae, plerumque ex ramis rectis vel pravis 2–60 μ longis et 3–6 μ latis apice ortae, rarenter paulum (5–25 μ) infra apicem rami ejusdem modi interpositae, etiam rarenter ex hyphis longis a latere oriundae vel in hyphas longas intercalatae.

Mycelium sparse but often extensive; mycelial hyphae colorless, continuous, sparingly branched, usually 3 to 6 μ wide, by means of an adhesive secretion at first colorless but later turning golden yellow capturing motile nematodes, then penetrating the integument of each captive with an infective tube that gives rise to assimilative branches

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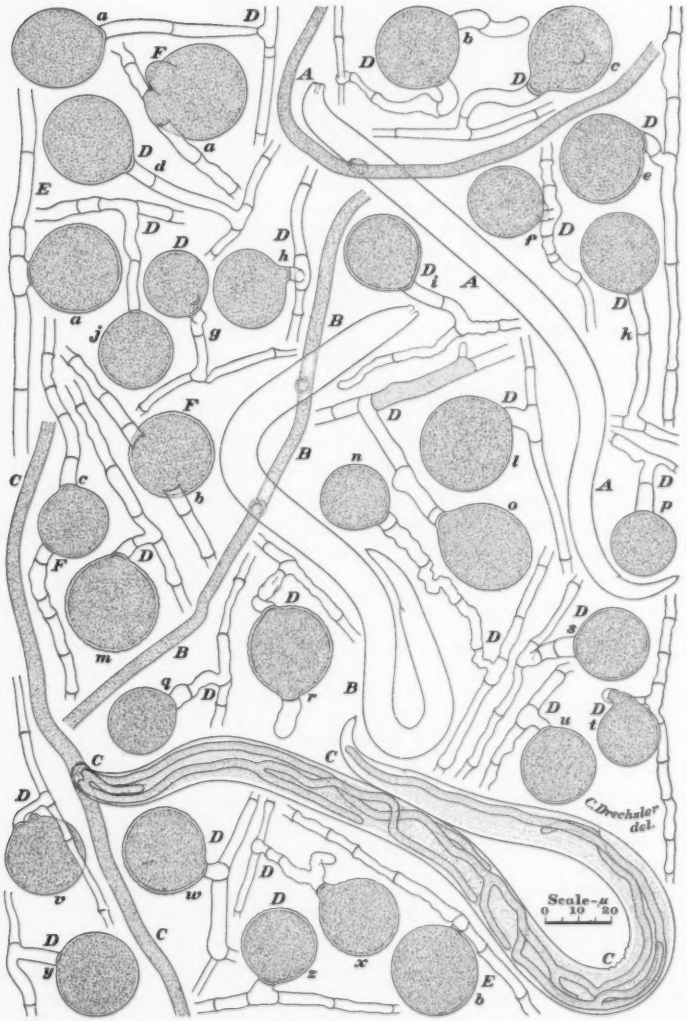

FIG. 1, *A–F*.

FIG. 1. *Cystopage cladospora* as found in maize-meal-agar plate cultures between 57 and 63 days old; × 500 throughout. *A, B*. Portions of mycelial hyphae, each with a newly captured specimen of *Plectus parvus*. *C*. Portion of hypha from which a captured specimen of *Plectus parvus* has been invaded by assimilative

commonly 2 to 3 μ wide, which appropriate the fleshy contents; chlamydospores at maturity usually yellowish, globose or ellipsoidal, commonly 20 to 30 μ in diameter, filled with densely granular contents, surrounded by a wall that is first smooth but later often becomes noticeably undulated, usually borne singly and terminally on straight or somewhat crooked branches mostly 2 to 60 μ long and 3 to 6 μ wide, occasionally borne intercalarily on such branches 5 to 25 μ from the tip, and occasionally, too, formed in lateral or intercalary position on the main mycelial hyphae.

Capturing and consuming nematodes of many species (including *Plectus parvus* Bastian) it occurs on decaying roots of various herbaceous plants and in decaying leaves of deciduous trees (*Quercus, Acer*) near Beltsville and College Park, Maryland.

Cystopage cladospora was first observed in maize-meal-agar plate cultures which after being overgrown by *Pythium ultimum* Trow had been further planted with small quantities of leaf mold from deciduous woods near Beltsville. It was found also in some Petri plate cultures prepared for the isolation of pathogenic oomycetes from decaying roots of lamb's quarters (*Chenopodium album* L.) and sheep sorrel (*Rumex acetosella* L.) collected on the grounds of the Plant Industry Station on August 18, 1955. All drawings of the fungus given in FIG. 1 were made between December 7 and December 13, 1955, from Petri plate cultures that had been planted on October 11, 1955, with discolored clearweed (*Pilea pumila* (L.) Gray) roots gathered near College Park. As the 2-month-old cultures had been protected against evaporation by being kept tightly covered with a battery jar, the substratum at the time was still moist and soft, permitting ready locomotion of the numerous eelworms present, most of which seemed referable to *Plectus parvus*. Capture of the animals by living hyphae of the fungus was still continuing actively, though the extensive distribution of well-matured chlamydospores on emptied tracts of mycelium showed that destruction of eelworms and reproductive development must have been proceeding for many days.

The mycelial hyphae of *Cystopage cladospora*, like those of *C. lateralis* and *C. intercalaris*, are among the widest produced by animal-capturing members of the Zoopagaceae. In extending radially from decaying

branches extending from head to tail. *D*. Portions of mycelium, *a–z*, each showing a chlamydospore borne on a short lateral branch; in two instances, *b* and *r*, the chlamydospore is found in subterminal position; in all other instances it is borne terminally. *E*. Portions of main hyphae, *a* and *b*, each with a lateral chlamydospore. *F*. Portions of main mycelial hyphae, *a–c*, each showing an intercalary chlamydospore.

material planted on a suitable agar substratum they show haphazard directional deviations in greater measure than mycelial filaments of *C. lateralis*. Capture of eelworms is effected by them through adhesion of the animals usually in 1 (FIG. 1, *A*) or 2 places (FIG. 1, *B*), but in some instances an animal is found affixed in 3 or 4 places. After narrow penetration of its integument in the adhering area the captive is invaded throughout by assimilative branches (FIG. 1, *C*), which become clearly discernible only when the materials resulting from the destruction of musculature and organs have largely been absorbed by them and conveyed backward into the parent mycelial filament. After the animal's fleshy contents have been wholly expropriated and the assimilative branches have been evacuated through progressive withdrawal of their protoplasm into the parent hypha, the empty integument of the nematode and the empty tubular membranes within it fade completely from sight.

Cystopage cladospora is distinguished from *C. lateralis* and *C. intercalaris* by its production of chlamydospores mainly on short lateral branches (FIG. 1, *D, a–z*). Most often the chlamydospores are formed terminally on the branches (FIG. 1, *D, a, c–q, s–z*) but in scattered instances they develop subterminally (FIG. 1, *D, b, r*). Development of chlamydospores laterally on the main hyphae—a feature especially characteristic of *C. lateralis*—can be observed only rarely in *C. cladospora* (FIG. 1, *E, a, b*). Their intercalary development in the main hyphae (FIG. 1, *F, a–c*) after the manner usual in *C. intercalaris* is likewise uncommon in the present fungus. To supply the growing reproductive body, a lengthy adjacent stretch of mycelial filament becomes progressively emptied, and, as in related species, retaining walls, marking successive stages in the withdrawal of protoplasm, are laid down at intervals. When newly formed, the chlamydospores of *C. cladospora* have a smoothly rounded outline (FIG. 1, *D, a, b, d*), but after several days they often present a noticeably undulated contour (FIG. 1, *D, m, o, r*). If, as seems probable, the wrinkling of the wall, or of an outer layer of the wall, results from shrinkage of the globose body, the shrinkage cannot well be attributed to desiccation. In the clearweed-rootlet cultures the chlamydospores acquired undulated sculpture while they were imbedded in agar moist enough to permit lively movement of eelworms.

Because of the less common hyphal relations of its chlamydospores *Cystopage cladospora* may be considered more distinctive than *C. intercalaris, C. ellipsospora* and *C. sphaerospora*. The new species would seem, however, not quite as distinctive or quite as readily identifiable as *C. lateralis*, which it was intended to establish as type of the genus by having its diagnosis directly follow the generic diagnosis. Since

according to a recent version of the rules governing botanical nomenclature (**4**) such arrangement of text has no relevant merit, occasion is here taken belatedly to designate *C. lateralis* as type species of *Cystopage*.

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MARINE FUNGI. III. PHYCOMYCETES

T. W. JOHNSON, JR.[1]

(WITH 22 FIGURES)

To the pioneer work of Petersen (**1905**) and Sparrow (**1934**) on marine Phycomycetes have recently been added extensive studies by Aleem (**1953**) and Kobayashi and Ookubo (**1953, 1954**). Höhnk (**1939, 1953**) and Höhnk and Aleem (**1953**) have contributed extensively to our knowledge of brackish-water Phycomycetes. Largely as the result of Sparrow's study (**1936**), the marine Phycomycetes of the Northeastern seaboard of the United States are well-known. A report of *Rhizophydium polysiphoniae* by Martin (**1922**) and the description of *Anisolpidium ectocarpii* by Karling (**1943**) apparently constitute the only additional records of algal-inhabiting marine Phycomycetes from the East Coast. The present paper is a report on some marine Phycomycetes collected in the Beaufort Inlet region, Beaufort-Morehead City, North Carolina, during the spring and summer of 1955 and 1956.

Paucity of material and failure to observe planonts and other critical phases in development, have prevented positive identification of several fungi. In the interest of completeness, however, these fungi are described insofar as possible.

Collections of marine algae on or in which the Phycomycetes were observed, were generally made from rock jetty habitats, or by scraping attached algae from buoys and driftwood debris. Hydrometric salinity determinations were made at each collection site, both at low and high tide. The lowest salinity determined was 33.6‰. Collections were examined within 24 hours after they were made, or after temporary storage at 5° C.

FUNGI COLLECTED

Chytridium polysiphoniae Cohn, Hedwigia **4**: 169. 1865. (FIG. 19.) Smooth-walled, hyaline, spherical or nearly spherical sporangia, 25–46 μ in diameter, mark this as a variant of Cohn's species. The Beaufort

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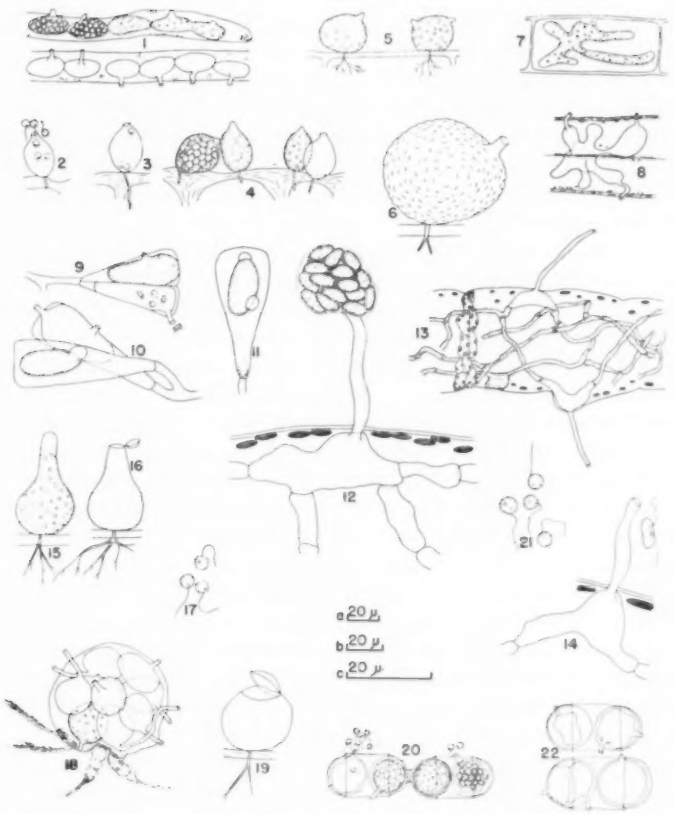

FIG. 1. *Sirolpidium bryopsidis*. 1. Immature thallus and discharged sporangia. FIGS. 2–4. *Phlyctidium brevipes* var. *marinum*. 2. Discharging sporangium. 3. Discharged sporangium showing long, simple rhizoid. 4. Sporangia. FIGS. 5, 6. *?Rhizophydium* sp. 5. Immature sporangia. 6. Discharged, crenulate sporangium. FIGS. 7, 8. *Petersenia lobata*. 7. Immature thallus. 8. Discharged sporangial thallus. FIGS. 9–11. *?Olpidiopsis* sp. 9. An immature and a discharging sporangium. 10. Discharged sporangium and resting spore. 11. Resting spore showing companion cells. FIGS. 12–14. *Lagenidium* sp. 12. Discharged sporangium showing spore vesicle. 13. Thallus and sporangia. 14. Empty sporangium and planont. FIGS. 15–17. *Chytridium* sp. 15. Immature sporangium. 16. Discharged, operculate sporangium. 17. Planonts. FIG. 18. Parasite in ostracod. 18. Sporangia. FIG. 19. *Chytridium polysiphoniae*. 19. Empty, operculate sporangium. FIGS. 20–22. *Olpidium entophytum*. 20. Discharging sporangia. 21. Planonts. 22. Discharged sporangia. FIGS. 2–4, 9–11, scale a; FIGS. 6, 12, 14–17, 21, scale c; others, scale b.

material differs from the fungus described by Cohn principally in sporangial shape. The operculum is apical, as Sparrow (**1943**) noted.

Petersenia lobata (Petersen) Sparrow, Dansk Bot. Arkiv **8**: 13. 1934. (FIGS. 7, 8.) This fungus is represented by a very sparse collection which was found in dead cells of *Ceramium*. Some thalli superficially resemble *Petersenia pollagaster*, also described from *Ceramium*. The sporangia are not, however, as deeply and broadly lobed as those of *P. pollagaster*.

Sirolpidium bryopsidis (deBruyne) Petersen, Oversigt Kgl. Danske Vidensk. Selskabs. Forhandl. **1905**: 480. 1905. (FIG. 1.) The planonts were not observed hence the identification may be considered questionable. However, the sporangia are remarkably similar to those in the description given by Sparrow (**1943**, p. 644), and the substratum (*Cladophora*) is the same. The Beaufort material bears a striking resemblance to *Olpidium aggregatum* Dangeard, a questionably valid taxon (Sparrow, **1943**, p. 97).

Thraustochytrium proliferum Sparrow, Biol. Bull. **70**: 259. 1936. Observations on planont and secondary sporangium development, and a description of the resting spores of this fungus, are to be published elsewhere.

Olpidium entophytum (Braun) Rabenhorst, Flora Europaea Algarum **3**: 283. 1868. (FIGS. 20–22.)

Sporangia spherical, subspherical, or saccate, predominantly slightly irregular; thin-walled; 12–27 μ in diameter. Discharge tube single, rarely two on a sporangium, isodiametric, projecting beyond host wall. Planonts globose, hyaline, with a single, colorless, refractive globule, and occasionally a dense granular body; posteriorly uniflagellate; 2.8–4.2 μ in diameter; at discharge temporarily quiescent at the exit orifice. Resting spores not observed.

In *Melosira* sp., rock seawall, Piver's Island, Beaufort Channel, Beaufort, North Carolina, August 7, 1956.

The identification is tentative, principally because the paucity of material prevented cross-inoculation studies. Planont size, sporangial size, isodiametric discharge tubes, mode of planont discharge, and, to some extent, sporangial shape, are features in which the Beaufort material resembles Braun's fungus. There is, however, a marked difference in substratum and habitat between the two, *O. entophytum* having been found previously on fresh-water green algae, the Beaufort collection in a marine diatom. Furthermore, the marine fungus has slightly irregular sporangia (FIG. 22), a characteristic not reported for *O. entophytum*.

Identification will turn on what future work may show to be most

significant in species delimitation, substratum and habitat or morphology. Five marine species of *Olpidium* have been described (all are considered questionable members of the genus by Sparrow, **1943**), but the fungus in *Melosira* appears to have less affinity to the marine forms than to the fresh-water forms. The possible exception is *O. entosphaericum* (Cohn) Fischer, but this species is too poorly described and inadequately illustrated to justify a comparison.

Phlyctidium brevipes var. *marinum* Kobayashi & Ookubo, Bull. Nat. Sci. Museum, Tokyo (N.S.) **1**: 62. 1954. (FIGS. 2–4.) There are certain marked differences between the Beaufort collection (on *Gracilaria*) and the fungus described by Kobayashi and Oukubo from *Bryopsis* sp. Outstanding among these are sporangial shape and prominence of the discharge papilla. The exit papillae of the fungus on *Gracilaria* are pronounced, agreeing in this respect with *P. brevipes* var. *brevipes* (Atkinson) Minden, but not with the variety *marinum*. Globose or subglobose sporangia seem to predominate in *P. brevipes* var. *marinum*, whereas the form on *Gracilaria* (judging from the very sparse material) has primarily elipsoid sporangia.

Chytridium sp. (FIGS. 15–17).

Sporangium epibiotic, sessile, pyriform with an attenuated apex, smooth-walled, hyaline; 26–32 × 18–21 μ; operculate. Rhizoids extensive, delicate, branched, endobiotic. Planonts posteriorly uniflagellate, spherical, with a hyaline, eccentric globule; escaping by dehiscence of an apical operculum and swimming away immediately; 2.8–4.2 μ in diameter.

Three sporangia were found on decaying *Ceramium;* the foregoing description is based on this very sparse collection. The fungus suggests *Chytridium polysiphoniae* in some characteristics, the most notable exception being that of sporangial shape. *Chytridium polysiphoniae* apparently does not produce sporangia with an attenuated apex, whereas the Beaufort material has distinctly long-pyriform sporangia. The collection is too sparse to justify more specific identification.

?Rhizophydium sp. (FIGS. 5, 6). Several eucarpic, epi-endobiotic sporangia were found on *Polysiphonia,* collected on Sharkshoal Jetty, August 3, 1955. Some sporangia were apparently immature, but more commonly the sporangia were discharged. Planonts were not observed nor could the immature sporangia be induced to mature. The sporangia are subglobose, hyaline, and have a densely crenulate wall (FIG. 6). One, rarely 2, subapical discharge tubes are produced. The very delicate, branched rhizoids are endobiotic. The sporangia measure 25–31 μ long by 22–25 μ in diameter, and are sessile on the host wall.

A branched, endobiotic rhizoidal system is the only feature of note

which suggests the genus *Rhizophydium,* and certainly excludes *Phlyctidium* as this genus is presently understood. The sporangia appear to be inoperculate, a feature which would exclude the fungus from *Chytridium.*

The Beaufort fungus differs markedly from the marine *Rhizophydium, R. codicola* Zeller, especially in the sporangial wall, which, in *R. codicola,* is smooth. *Rhizophydium verrucosum* Cejp (on *Closterium* sp.) differs from *Rhizophydium* sp. principally in sporangial shape, but is similar to some extent in wall ornamentation. *Rhizophydium* sp. lacks the prolonged apiculus described for *R. verrucosum.* Until more abundant material is available for study, *Rhizophydium* sp. must remain a fungus of undetermined affinities.

Lagenidium sp., parasitic (?) in *Gracilaria.* (FIGS. 12–14.) The *Gracilaria* in which the fungus was found was collected on a small segment of *Spartina* stem dredged from 25 feet of water, 1½ miles off Bogue Banks, North Carolina, July 28, 1956. A single filament of *Gracilaria* was infected.

The vegetative thallus is extensive, consisting of sparingly-branched, slender hyphae. The hyphae are usually constricted at the septa. The sporangia are variable in shape, and are apparently formed by expansion or swelling of portions of the sparsely septate thallus. Generally, the sporangia measure 28–35 μ long by 14–17 μ in diameter, but may reach a length of 42 μ. A single, long, slightly irregular discharge tube is produced, at the apex of which, at discharge, a vesicle forms. Spores are cleaved out within this vesicle and subsequently emerge as laterally biflagellate, reniform planonts. The discharge tube is not contricted at its point of passage through the algal wall. Resting spores were not observed.

This *Lagenidium* falls readily into the group of species which have extensive, myceloid thalli, that is, *L. closterii, L. marchalianum,* and *L. giganteum,* but differs from these species principally in sporangial shape. In *Lagenidium* sp. the sporangia are clearly distinct and definite thallus segments which, when mature, do not retain the narrow tubular shape of the thallus segments. In *L. closterii,* for example, the sporangium is narrowly tubular with a large spherical or subspherical swelling in the basal portion of the discharge tube below the host wall. In *Lagenidium* sp., on the other hand, the sporangium is a definite swollen segment, and the discharge tube is not expanded basally. Contrary to the condition of planont cleavage before discharge as occurs in *L. marchalianum,* the planonts of *Lagenidium* sp. are completely cleaved in the vesicle at the apex of the discharge orifice. A single marine

species, *L. callinectes,* is known (Couch, **1942**). The fungus in *Gracilaria* differs from *L. callinectes* in sporangial shape, shape and extent of the discharge tubes, and in thallus texture. The thallus of *L. callinectes* is much stouter than that of the present fungus.

The very distinct sporangia mark *Lagenidium* sp., and suggest an advanced degree of specialization toward the filamentous biflagellate Phycomycetes. Established species such as *L. giganteum* exhibit a complexity of thallus and sexual reproductive organ morphology quite suggestive of certain species of *Pythium. Lagenidium* sp., by reason of the distinct sporangia, bears a definite resemblance to pythiaceous fungi.

Lagenidium sp. is clearly an undescribed form. The fungus is not described as a new species at this time because of the paucity of material, most of which was inadvertently discarded. Furthermore, since the asexual morphology is so suggestive of that of a *Pythium,* it seems inadvisable to erect a new species until such time as the sexual reproductive stages are observed.

?Olpidiopsis sp., parasitic in *Licmophora.* (FIGS. 9–11.) Two colonies of *Licmophora,* occurring on *Ectocarpus,* were infected by an Olpidiopsis-like fungus. The host alga was very sparsely infected, and repeated attempts to find other infected material of the same host in the same locality failed.

The sporangia are generally ellipsoid or ellipsoid-conic, with few exceptions the shape being determined by that of the diatom frustules. Usually, only one sporangium is produced in a single diatom, but two and three may occur rarely. When mature, the smooth, thin-walled sporangia measure 28–54 μ long by 18–39 μ in diameter. One or two short, narrow discharge tubes are produced, and, as near as could be determined, these either penetrate the host wall, or force apart the valves of the host without penetration. The sporangia are very similar to those of *Ectrogella perforans,* occurring in the same host. The discharge tubes of *E. perforans* are, however, broad-conic, whereas those of the *Olpidiopsis* are narrow-cylindric. Discharge of the laterally biflagellate planonts in both fungi follows a similar pattern. In a few host plants, either those of the same colony in which sporangia were present, or in separate colonies, hyaline, smooth-walled, ellipsoid or spherical resting spores occurred. No instances of occurrence of resting spores and sporangia in the same infected host cell were observed. The resting spores measured 22–37 μ long by 15–25 μ in diameter. One or two hemispherical companion cells were present on about one-third of the resting spores which were examined. In all instances, the com-

panion cells were without protoplasmic content. Germination of the resting spores was not observed.

The nature of the resting spore suggests that the fungus in *Licmophora* is an *Olpidiopsis*. Inasmuch as companion cells were not observed on all resting spores, however, the relation is less certain. Sparrow's observation of a small "cyst" on one resting spore of *Ectrogella perforans*, although not conclusive evidence, suggests that there is a very close similarity between *Ectrogella* and *Olpidiopsis*. Based on present knowledge of these two genera, however, the evidence from the morphology of the present fungus is more in favor of the latter genus.

Among the known species of *Olpidiopsis*, the fungus in *Licmophora* is nearest *O. andreei*, which occurs in filamentous marine algae. The principal differences, aside from host, between *Olpidiopsis* sp. and *O. andreei*, are 1) fewer discharge tubes in the former, and 2) fewer sporangia in any one host cell in the case of *Olpidiopsis* sp. The latter feature may be attributable entirely to host cell size. Aside from host difference and predominant shape of the sporangia, the characteristics of *Olpidiopsis* sp. compare favorably with those of *O. sphacellarum*, also occurring in marine algae, but not in diatoms. Since the resting spores of *O. sphacellarum* (a questionable member of the genus according to Sparrow) are not known, there is no conclusive evidence that the fungus in *Licmophora* is the same as *O. sphacellarum*.

The final position and identification of *Olpidiopsis* sp. must await cross-inoculation studies, a phase of investigation not possible with the very sparse material at hand. The evidence is suggestive, but by no means conclusive, that the Beaufort fungus is a variant of *Ectrogella perforans*.

Parasite (?) in ostracods. (FIG. 18.) This fungus was found in a population of an unidentified ostracod collected in a mass of decaying algae and silt scraped from the undersurface of a sand dollar test (*Mellita quinquiesperforata*). The sporangia, only one of which was undischarged, varied from 3 to 11 in number in the infected animals. Each sporangium produces a single discharge tube extending beyond the host wall. Most of these tubes show a slight constriction at the point where the tube penetrates the wall. Ovoid or slightly irregular sporangia, 20–28 × 15–20 μ, predominate, with a few spherical ones measuring 17–25 μ in diameter occasionally being produced. Planonts and resting spores were not observed.

The affinities of this fungus are unknown. Morphologically, it resembles *Olpidium* or *Olpidiopsis*, but this can be confirmed only by

observation of planont flagellation. There is some evidence, based entirely on supposition from a single observation, that the fungus may be a species of *Pontisma*. In two infected animals a short, segmented, densely protoplasmic thallus was observed; the segments were few in number, but of a size consistent with that of the discharged sporangia. Maturation of these segments was not observed, and, in fact, they may not have been developmental stages of the fungus producing the sporangia. As far as is known, *Pontisma* is restricted to the marine alga *Ceramium*. None of the marine phycomycetous fungi known to occur on marine crustacea (Sparrow, **1943**, p. 721) bear any close resemblance to the one in the ostracods.

SUMMARY

The marine Phycomycetes *Chytridium polysiphoniae, Petersenia lobata, Sirolpidium bryopsidis, Thraustochytrium proliferum,* and *Phlyctidium brevipes* var. *marinum* are reported from the North Carolina coast. *Chytridium* sp. and *Lagenidium* sp. are described. Two unnamed forms are discussed and tentatively assigned to the genera *Olpidiopsis* and *Rhizophydium*. The sporangial stage of an endobiotic, holocarpic fungus occurring in ostracods is described, but the generic position is not established. An *Olpidium*, tentatively identified as *O. entophytum*, is reported from *Melosira*.

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A NEW SPECIES OF TRICHOPHYTON FROM NEW SOUTH WALES [1]

E. Beatrix Durie and Dorothea Frey

(with 8 figures)

Recent work on the isolation of pathogenic fungi, including dermatophytes, has been briefly reviewed in a previous paper (**4**). In the course of investigating a series of samples of soil for the presence of keratinophilic fungi, a new species of *Trichophyton* has been encountered. It was isolated from two different samples of soil by a technique described by Vanbreuseghem (**8**) and modified by Ajello (**1**).

Trichophyton terrestre sp. nov.

Culturae in agaro Sabouraudi sunt albae et lanosae in margine, sulphureae et granulosae in centro. Microconidia piriformia, 4–6 × 3–5 μ in latere feruntur, aliquando in uvis. Macroconidia ovalia ad clavata, 8–30 × 4–5 μ, parietibus tenuis et levibus, duoseptata ad sexseptata sunt, plerumque ovalia et duoseptata. Formae etiam sunt inter microconidia et macroconidia. Noduli multiplices cum hyphis similibus cornibus cervi videntur. Ex humo Australiense. Innoxium cavibus.

The literature contains very little reference to the saprophytic existence in soil of dermatophytes other than *Microsporum gypseum* and *Keratinomyces ajelloi*.

Trichophyton mentagrophytes is mentioned only twice, so far as we know, in this connection. Muende and Webb (**7**) found *Trichophyton mentagrophytes* in a shed where infected animals had been kept. The same fungus was isolated from the soil of two caves in the Transvaal by Lurie and Borok (**6**).

MATERIALS AND METHODS

One of the soil samples which yielded the new isolate was collected from the side of a country road running between bush on the one side and farm-paddocks on the other near the village of Appin, New South Wales; the other from a garden adjoining bushland at the mountain village of Wentworth Falls, New South Wales. The procedure em-

[1] This work was supported by a full-time grant to one of us (D. F.) from the National Health and Medical Research Council of Australia.

ployed was the same as in our previous work (**2**, **4**). Petri dishes half-filled with soil were baited with autoclaved human hair and examined at weekly intervals. After four weeks there was a moderate growth of white downy mycelium with minute white nodules on the hairs. These hairs were transferred to five different media; namely, Sabouraud's glucose agar containing 0.5 mg of "Actidione" (cycloheximide); 20 units of penicillin and 40 units of streptomycin per ml; potato-dextrose agar; corn-meal agar; rice medium, and blood agar containing 0.2 μg of thiamin per ml.

FIG. 1. *Trichophyton terrestre*. Petri plate containing soil, showing appearance of fungus on human hairs used as bait. Slightly reduced.

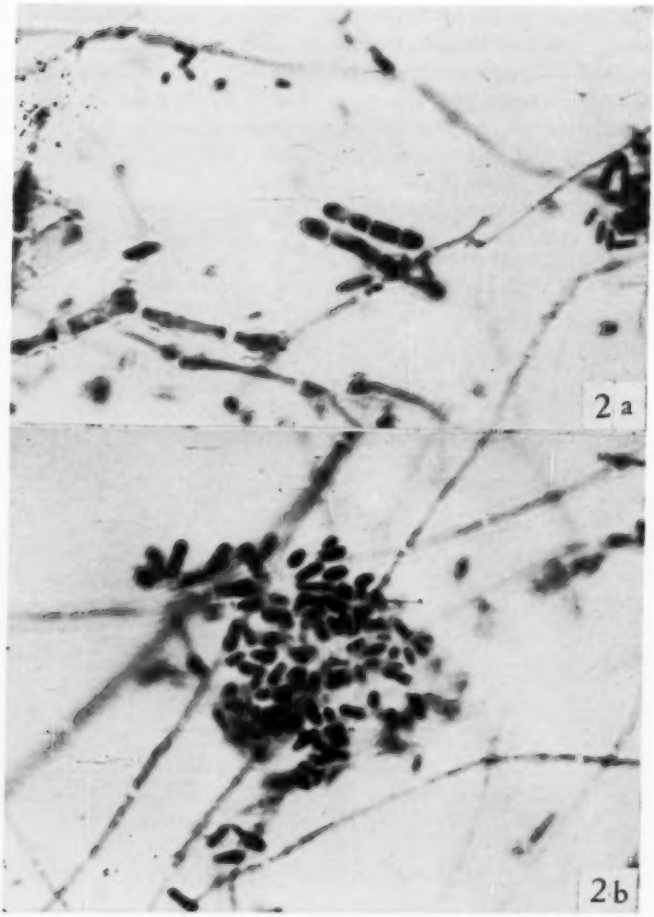


FIG. 2. *Trichophyton terrestre.* a. Macro- and microconidia in 4 weeks culture, on Sabouraud's agar with cycloheximide, × 1000. b. Microconidia and intermediate forms in 4 weeks culture, × 1000.

RESULTS

On the soil plate after 4 weeks the hairs were covered with white downy mycelia with many small white dots (FIG. 1). Microscopic examination showed hairs surrounded by a mycelial growth with many slender, several-celled, clavate, smooth and thin-walled macroconidia;

masses of single-celled pyriform microconidia en grappe and en thyrses; septate hyphae and racquet mycelium. The attacked portions of hair as well as the fungus were deeply stained by cotton-blue in a plastic mountant. The macroconidia varied widely in size; the majority were ovoid and had only one or two septa; intermediate forms between the macroconidia and microconidia were seen (FIG. 2).

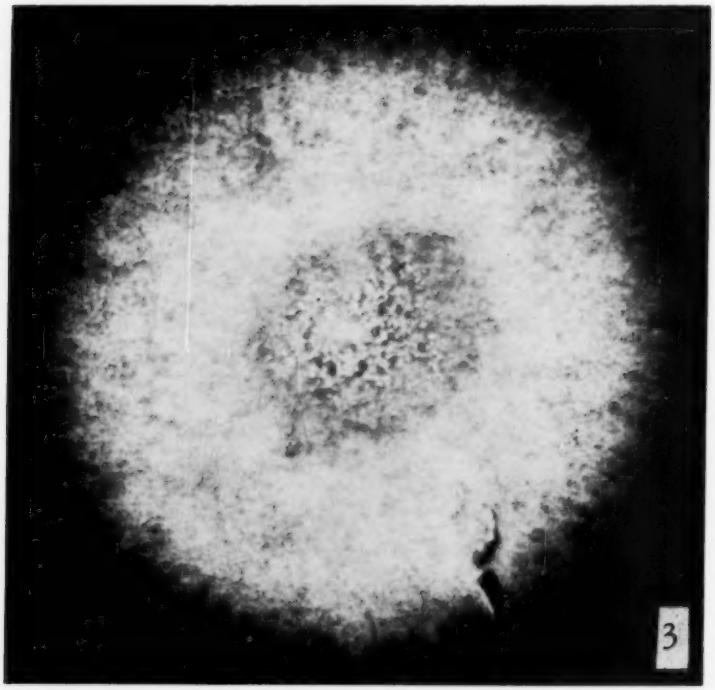


FIG. 3. *Trichophyton terrestre.* Grown in Petri plate on Sabouraud's glucose agar with cycloheximide, penicillin and streptomycin, 14 days, × 2.

GROWTH ON OTHER MEDIA

Macroscopical examination: On Sabouraud-cycloheximide-penicillin-streptomycin agar, the moderately fast-growing fungus (40 mm in diameter, 13 days) was at first white and fluffy with a pale yellow granular center. The reverse of the colony showed a yellowish brown color. With age the colony changes to a velvety appearance (FIG. 3).

On potato-dextrose agar the moderately fast-growing colony (35 mm in diameter, 10 days) appeared granular with white fluffy edges and radiating strands. The reverse of the colony was yellowish (FIG. 4).

On corn-meal agar the fungus displayed a scanty growth (10 mm in diameter, 12 days). Colony velvety; the reverse of the colony was pale yellow.

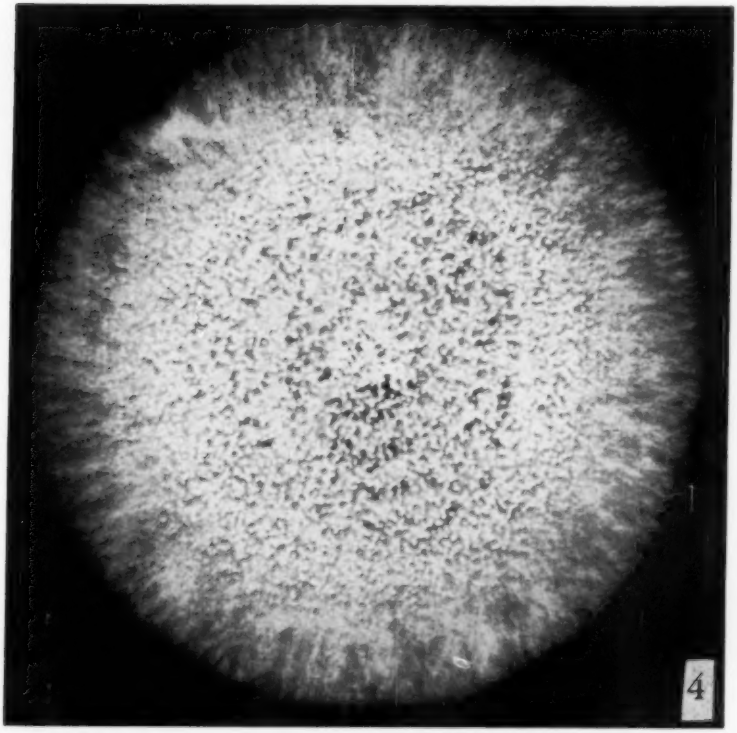
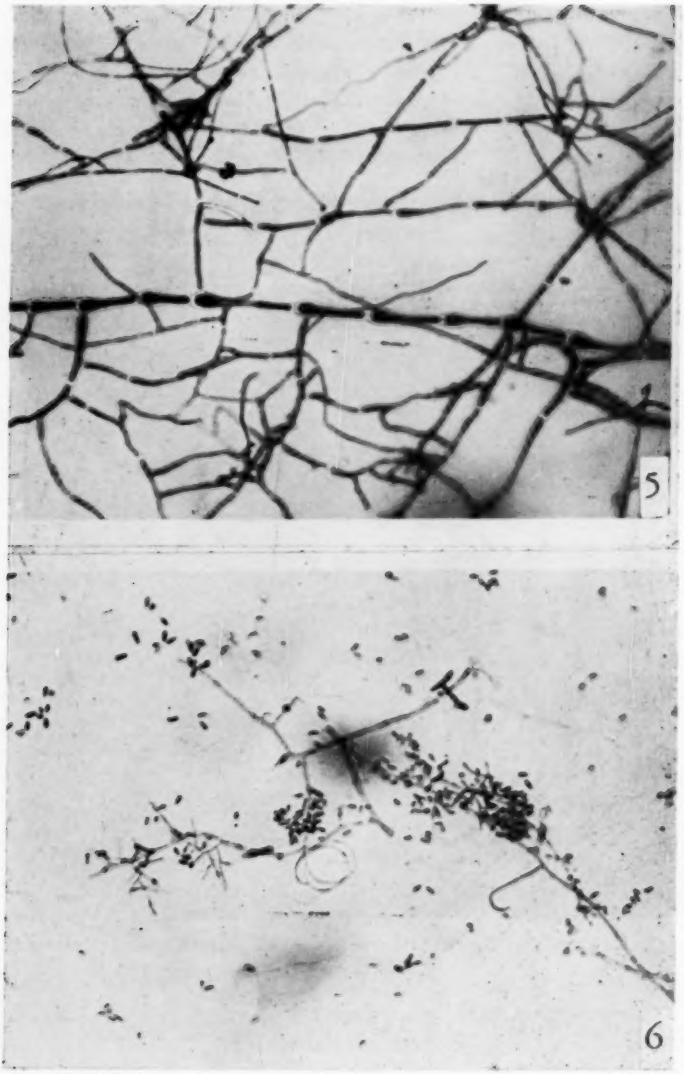


FIG. 4. *Trichophyton terrestre.* Grown in Petri plate on potato dextrose agar, 14 days, × 2.

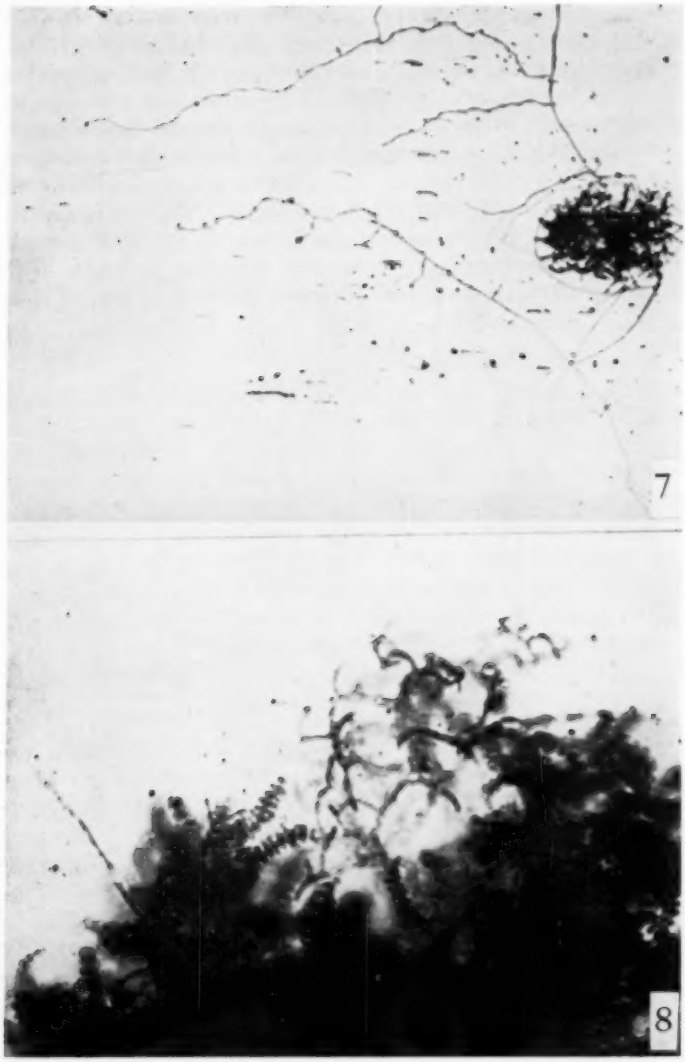
On rice medium no characteristic colony was formed. Grains were covered with a yellowish green felt-like mat.

On blood-thiamine agar the moderately fast-growing colony (35 mm in diameter, 10 days) was white and velvety in appearance.

Microscopical examination: The vegetative mycelium was divided by septa into segments of approximately 20 to 30 μ in length. The proto-



FIGS. 5, 6. *Trichophyton terrestre*. 5. Slide culture on cycloheximide agar, showing racquet mycelium, 10 days, × 400. 6. Slide culture on cycloheximide agar showing spiral, microconidia en grappe and occasional macroconidia, 12 days, × 200.



FIGS. 7, 8. *Trichophyton terrestre*. 7. Slide culture on corn-meal agar, showing nodular complex, 14 days, × 200. 8. Culture mount from nodule on soil plate showing antler-like hyphae and encrusted hyphae, 4 weeks, × 400.

plasm was often seen somewhat withdrawn from the cell wall. The vegetative mycelia gave rise to aerial mycelia which produced macroconidia and microconidia. The macroconidia were clavate, smooth and thin-walled, sessile and 2–6-celled. They measured 8–30 × 4–4.8 μ. The microconidia appeared pyriform, en grappe and en thyrses, and measured 4–6.4 × 3.2–4.8 μ. Intermediate forms between macroconidia and microconidia were observed. The number of these structures varied somewhat with the different media employed. They were especially abundant on rice medium and on blood-thiamine agar. Terminal and intercalary chlamydospores, spirals, racquet hyphae, antler-like hyphae and nodular organs were occasionally also observed (FIGS. 5–8).

PATHOGENICITY TESTS

Attempts to produce lesions in four guinea-pigs by scarification and in two by inoculation were unsuccessful in our hands. Dr. C. W. Emmons (personal communication), who has kindly examined this fungus for us, informed us that two attempts to produce experimental dermatophytosis were unsuccessful. Professor Vanbreuseghem (personal communication) was also good enough to examine this fungus for us and found it non-pathogenic for guinea-pigs. There is thus fair evidence of non-pathogenicity, especially as, according to Emmons, these granular strains are usually quite virulent. It is realized that further pathogenicity tests should be performed; this will be done later.

PRODUCTION OF TRICHOPHYTIN

Following a method described by Langeron and Vanbreuseghem (**5**), a culture of this fungus in 250 cc of nutrient broth containing 2% glucose in a 500 cc Erlenmeyer flask was incubated at room temperature for three months, Seitz filtered and checked for sterility. Intradermal tests on four normal guinea-pigs showed no reaction. Intradermal tests using this extract in parallel with a commercially prepared "Trichophytin" (Hollister-Stier laboratories, Los Angeles, California) were then performed on seven human subjects, four of whom had had Trichophyton infections proved by culture. The results are shown in the accompanying table.

DISCUSSION

Trichophyton terrestre closely resembles the dermatophytes and in spite of some pecularities its appearance seems to justify its inclusion in the genus *Trichophyton*. It produces spirals, nodular organs, micro-

and macroconidia, chlamydospores and antler-like hyphae (Emmons, **3**) typical of that genus. It is keratinophilic and keratinolytic. *In vitro* it destroys hair but does not show the "perforating organs" shown by *Microsporum gypseum* and *Keratinomyces ajelloi.* An extract prepared in the way recommended for the production of "Trichophytin" (**5**), when injected intradermally, produced cutaneous hypersensitive reactions in four human subjects with proven *Trichophyton* infections and one subject with suspected infection. No such reaction occurred in two uninfected persons nor in normal guinea-pigs. So far the fungus

TABLE I

Initials of subject	History as regards infection with *Trichophyton*	Reaction to intradermal injection of extract from *Trichophyton terrestre*		Reaction to commercial "Trichophytin" Diluted 1 in 30
		Undiluted	Diluted 1 in 30	
D. F.	None	−		−
E. B. D.	None	−		−
A. W.	Tinea pedis suggestive of *Trichophyton* infection	++++		+++
B. G.	Tinea capitis. *Trichophyton sulphureum* grown.		+	+++
P. W.	Tinea pedis. *Trichophyton mentagrophytes* grown.		++	++
K. B.	Tinea cruris. *Trichophyton mentagrophytes* grown.		++++	+
D. E.	Tinea pedis. *Trichophyton mentagrophytes* grown.	[illegible]	+++	+++

+ Signifies erythema (0.5 cm in diam.) with slight swelling and induration.
++ Signifies erythema (0.7 cm in diam.) with moderate swelling and induration.
+++ Signifies erythema (2 cm in diam.) with corresponding swelling and induration.
++++ Signifies erythema (2.5 cm in diam.) with marked swelling, induration and desquamation.

seems to be non-pathogenic to guinea-pigs. Further pathogenicity tests should of course be done. Vanbreuseghem (**9**) described a keratinophilic fungus, *Keratinomyces ajelloi,* which was non-pathogenic for guinea-pigs but produced an extract which reacted like "Trichophytin." He suggested that side by side with the known dermatophytes there existed "dermatophytes non pathogènes." It is indeed not surprising to find that non-pathogenic variants of the dermatophytes exist in nature, for among the bacteria, which have been studied much more fully than the fungi, most genera which include pathogens include also some non-pathogenic species and in any pathogenic species virulent and non-virulent strains may occur. Resistance of the host of course plays a

part in this kind of classification. It is generally supposed that it is the pathogen, not the non-pathogen, which is the variant, and this is probably the case with the keratinophilic fungi. As Vanbreuseghem said (**9**), "L'existence dans le sol de dermatophytes non pathogènes tel que *Keratinomyces ajelloi*, doués d'un pouvoir keratinolytique important, nous explique d'une part la désintégration de substances naturelles à base de kératine (poils, cornes, plumes) et la destruction de tissus à base de fibres naturelles (lainages). Cette question est encore très obscure."

Morphologically, as the above description shows, this fungus must be placed in the genus *Trichophyton* and the production of a "Trichophytin" which reacts in parallel with a known "Trichophytin" seems to place it among the dermatophytes though not necessarily in any one genus. The species which it resembles most closely is *Trichophyton mentagrophytes*, but it differs from this species in the following respects: The rate of growth is more rapid and the colony larger and thicker; this at least was the case when this species was compared with two known strains of *Trichophyton mentagrophytes* grown in parallel on the five media mentioned above. The appearance of the colony, with its pale sulphur-yellow granular center, differs from that of any strain of *Trichophyton mentagrophytes* which we have encountered. This color is seen in *Trichophyton tonsurans* but the colony is usually folded, not granular. The macroconidia are more numerous than and different from those of *T. mentagrophytes*. Many have only one septum and are ovoid in shape. Intermediate forms between macroconidia and microconidia occur. The nodules or complexes seen on soil plates and sometimes on culture on artificial media have not been observed in cultures of *Trichophyton mentagrophytes* introduced onto sterile soil plates or grown on other media. Finally, although this is a granular strain, it has not been found possible to produce lesions in guinea-pigs.

SUMMARY

A new species of *Trichophyton* has been isolated from two samples of soil in the County of Cumberland, New South Wales, Australia. The organism is distinguished by the appearance of the colony and the abundance of small ovoid macroconidia. So far it appears to be non-pathogenic for guinea-pigs. It produces "Trichophytin."

ACKNOWLEDGMENTS

Several acknowledgments must be made, for very little work in mycology is being done in Australia, and we are very dependent on

help and advice from overseas. Our thanks are due to Dr. C. W. Emmons of the Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, U.S.A., for examining this culture for us and for help in the preparation of this paper; to Professor R. Vanbreuseghem, Institut de Médecine Tropicale Prince Leopold, Antwerp, who also examined this culture for us; to Dr. L. Ajello of the Communicable Disease Center, Chamblee, Georgia, U.S.A., who examined this culture and others for us and who has often given us help and advice in our work with soil fungi; and to Dr. W. Forbes and Mr. O'Keefe of the School of Public Health and Tropical Medicine, Sydney, for making the photographs shown in this paper.

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CERCOSPORA LEAFSPOT OF PHOTINIA SERRULATA

LOUIS ANZALONE AND A. G. PLAKIDAS

(WITH 1 FIGURE)

For many years nurserymen in Louisiana have had difficulty in propagating *Photinia serrulata* Lindl., an attractive and desirable ornamental shrub, because of a disease that attacks the foliage causing numerous necrotic spots and severe defoliation.

DESCRIPTION OF THE DISEASE

In the early stages, the lesions first appear as indefinite chlorotic spots, 1–2 mm in diameter, on both the upper and lower leaf surface. Soon the spots change to purple, increase in size to 2 to 25 mm in diameter, and finally become dark brown (FIG. 1). On the under surface of the leaf the spots are lighter brown in color and more indefinite in outline. The central portion of the spots on either leaf surface may appear downy or velvety due to the presence of large numbers of conidiophores and conidia. Soon after the spots develop over a large portion of the leaf, abscission takes place and partial defoliation of the plant results. Lesions on the new growth usually begin to appear early in June, and the disease increases in severity as the summer progresses so that, by the end of October, the plants are severely defoliated with only small tufts of leaves at the terminal portions of the shoots. The cause of this disease was found to be an apparently undescribed species of *Cercospora*. This fungus is here described and named.

IDENTITY OF THE CAUSAL FUNGUS

Three species of *Cercospora* have been reported on *Photinia*, namely *C. heteromeles* Harkness (Cal. Acad. Sci. Bul. **1**: 38. 1884) on *P. arbutifolia* (Ait.) Lindl., *C. eriobotryae* (Enjoji) Sawada (Catalogue of Formosan Fungi. Part VI. Rept. **61**: 94. 1933) on *P. glabra* (Thunb.) Maxim., and an undescribed species (Index of Plant Diseases in the United States, p. 952) on *P. serrulata* Lindl.

Although no direct comparison of specimens of *C. heteromeles* Harkness with the Louisiana *Cercospora* was made, it is safe to state that the

latter is definitely not *C. heteromeles.* The conidia of *C. heteromeles* are described as dark olivaceous with distinct septa and 5–7.5 μ in thickness; those of the Louisiana *Cercospora* are hyaline with indistinct septa and 1.9–2.8 μ thick. Fructifications of *C. heteromeles* are described as hypophyllous; those of the Louisiana Cercospora are definitely amphigenous. There are also other differences between the two fungi in the shape, size, color, and scar size of their conidiophores.

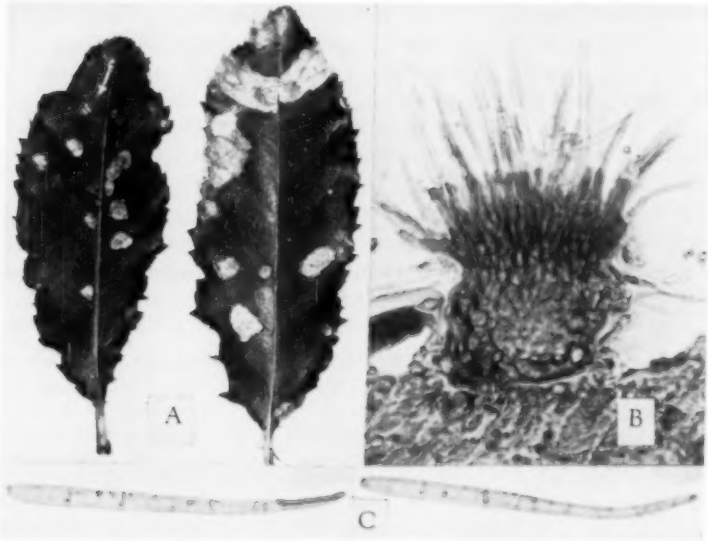

FIG. 1. *Cercospora photiniae-serrulatae.* A. Leaves with typical spots. B. Stroma with conidiophores and conidia, × 630. C. Conidia, × 1100.

There is considerable similarity between the Louisiana fungus and *C. eriobotryae* (Enjoji) Sawada in regard to shape and size of their fructifications and conidia. However, the two fungi are considered distinct for the following reasons: (1) The conidia of the Louisiana Cercospora are definitely hyaline; those of *C. eriobotryae* have been described [1] as "subhyaline to very pale olivaceous." (2) Host differences: *C. eriobotryae* has been reported as pathogenic on *Photinia glabra* and *Eriobotrya japonica.* Repeated inoculations of these two hosts with the Louisiana fungus (both with pure cultures and with conidia from

[1] In Chupp's "A monograph of the fungus genus *Cercospora.*" Original description not seen.

P. serrulata leaf lesions) failed to cause infection, although parallel inoculations on *P. serrulata* plants caused infection readily. Neither has natural infection ever been observed on *P. glabra* in Louisiana. In one nursery, in particular, where these two species were growing on adjacent rows, with their branches touching, it was observed that the foliage of all the *P. serrulata* plants was severely spotted while that of the *P. glabra* plants was completely healthy. Specimens of *C. eriobotryae* (Enjoji) Sawada, consisting of leaf pieces of *P. glabra* and *Eriobotrya japonica,* and obtained from the Cornell University herbarium, were examined. No fructifications were found on *E. japonica;* fructifications were present on *P. glabra,* but the conidiophores and conidia were in poor condition, not very suitable for comparison with the Louisiana *Cercospora.*

This brings us to consideration of the third species of *Cercospora* on *Photinia,* the one reported from Georgia, but apparently never described. The Louisiana fungus is identical with that in Georgia. We obtained fresh specimens of diseased *P. serrulata* leaves from Georgia, through the courtesy of Dr. Luther Farrar, for comparison and found both the disease and causal fungus to be identical with those in Louisiana. However, we have been unsuccessful in tracing the original report from Georgia of a *Cercospora* on *P. serrulata.* The only reference to this disease we have found is that in "Index of Plant Diseases in the United States," p. 952, where is listed "*Cercospora* sp. (not *C. heteromeles*) . . . Ga. (1, 3)."

Several botanists at the University and Agricultural Experiment Station in Georgia were queried concerning this reference, but no record was found as to when and by whom the report had been made. Dr. Charles Chupp of Cornell University was consulted for possible information on the Georgia report and he reported as follows: "I know nothing of the *Cercospora* sp. (not *C. heteromeles*) from Georgia. I went through the folders of the five or six botanists who corresponded with me about *Cercospora* from that state but found no mention of such a specimen." Dr. Paul R. Miller kindly informed us that the survey records of the Plant Disease Epidemics and Identification Section, U. S. Department of Agriculture, did not indicate the source of the reference to this disease in the "Index of Plant Diseases in the United States." It appeared, therefore, that this *Cercospora* on *P. serrulata* in Georgia and Louisiana was an undescribed species.

DESCRIPTION OF THE FUNGUS

The mycelium occurs internally in the form of branching hyphae. This internal mycelium is intercellular between the spongy parenchyma

and palisade cells. Fruiting is amphigenous but chiefly epiphyllous in the form of dark brown to black, globular stromata, which range in size from 21 to 105 μ in diameter. In general, the stromata are smaller on the lower leaf surface. The stromatic structures are embedded among the cells below the epidermal layer and emerge by breaking through it. The fascicles are very dense and compact (FIG. 1, B). The conidiophores are 2.6 to 3.9 μ in diameter at their thickest point, dark in mass, but singly pale to olivaceous brown, paler and narrower toward the tip, sparsely septate, occasionally branched, straight to slightly geniculate, with medium sized spore scars, and about 10 to 43 μ in length. The conidia are obclavate, hyaline, straight to mildly curved, 1–10-septate with septa indistinct, base obconically truncate, tip subacute. They are 1.9–2.8 μ in diameter and 29.9–99.3 μ in length.

The fungus was obtained in pure culture from tissue planting, and it grew well but slowly on potato dextrose and oatmeal agar media. The colonies on these media were compact, raised, light to dark gray on top and greenish black on reverse. The fungus did not sporulate on these media.

Cercospora photiniae-serrulatae sp. nov.

Maculis irregularibus, primo chloroticis, in colorem purpureum se gradatim immutantibus, postremo necroticis, in superficie superiore fusco-brunneis, in superficie inferiore subbrunneis. Fructificatione amphigena; magnis stromatibus, sub epidermide sursum surgentibus, erumpentibus; conidiophoris e stromate in fasciculis densis compactisque exsistentibus, pro magna parte haud ramosis, raro septatis, pallidis usque ad colorem olivo-brunneum, prope cucumina pallidioribus et angustioribus, erectis usque ad subgeniculata, cicatricibus magnitudine mediis, 2.6–3.9 $\times$ 10.4–42.9 μ; conidiis hyalinis, obclavatis, cacumine subacuto; basi obconico-truncata, rectis aut leniter curvatis, 1–10-septatis; septis indistinctis 1.9–2.8 $\times$ 29.9–99.3 μ.

Spots irregular, chlorotic specks at first, turning purple and finally necrotic, dark brown on the upper surface and lighter brown on the lower surface. Fruiting amphigenous; stromata large, arising subepidermally, erumpent; conidiophores arising in dense, compact fascicles from the stroma, mostly non-branched, sparsely septate, pale to olivaceous brown and paler and narrower toward the tips, straight to slightly geniculate, scars medium size, 2.6–3.9 $\times$ 10.4–42.9 μ; conidia hyaline, obclavate, tip subacute, base obconically truncate, straight to mildly curved, 1–10-septate, septa indistinct, 1.9–2.8 $\times$ 29.9–99.3 μ.

HOST: *Photinia serrulata* Lindl.

TYPE LOCALITY: Loranger, Louisiana, Aug. 7, 1956, L. Anzalone, No. *8164*.

DISTRIBUTION: The disease was observed in several localities in Louisiana (Folsom, Lecompte, Loranger, Baton Rouge, Plaquemine). Specimens were also received from Georgia.

Type specimens (No. *8164*) deposited in the mycological herbarium of the Department of Botany, Bacteriology, and Plant Pathology, Louisiana State University, Baton Rouge, Louisiana and also in the National Fungus Collections, Bureau of Plant Industry, Washington, D. C., and the herbarium of Cornell University, Ithaca, New York.

PATHOGENICITY AND HOST SPECIFICITY

The disease was reproduced in its typical form on *P. serrulata* by inoculations both with pure cultures (macerated mycelium) and with conidia from lesions on *P. serrulata* leaves. Parallel inoculations of *P. glabra* and *Eriobotrya japonica* failed to produce disease.

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CONIDIAL STAGE OF THE LICHEN FUNGUS BUELLIA STILLINGIANA AND ITS RELATION TO SPORIDESMIUM FOLLICULATUM [1]

MASON E. HALE, JR.

(WITH 1 FIGURE)

Conidia are represented in nearly all lichen genera by minute unicellular pycniospores (spermatia?) produced in typical pycnidia, rarely in the hymenium among paraphyses or even in asci (Johnson and Brown, **1941**). The function of these microconidia is unknown, although in some instances they can germinate and form a mycelium (Möller, **1887**). In a few species of tropical epiphyllous lichens these pycnidia contain unicellular pycniospores and/or large septate colorless "stylospores," or macroconidia (Müller, **1885**; Santesson, **1952**), similar to those of the imperfect fungus *Septoria*. Bornet's (**1873**) report of unicellular brown conidia in the symbiont stage of *Lempholemma minutulum* has not been confirmed.

Although the fungal components of approximately 70 species of lichens have been isolated, there are few convincing reports of conidial formation in pure culture. Möller (**1887**) in a pioneering study found conidia (oidia?) in *Calicium*, a genus closely related to the non-lichenized fungus *Roesleria*. Other early records of conidia appear to be based on contaminating imperfect fungi and have not been verified. Werner (**1927**) described unicellular conidia arising from aerial hyphae in cultures of *Cladonia squamosa*, but they are perhaps more properly classified as oidia. These conidia were not observed in later cultures of the species by Thomas (**1939**).

The writer recently discovered conidial formation in cultures of the corticolous crustose lichen *Buellia stillingiana*. The fungus was isolated

[1] This work supported in part by a grant from the National Science Foundation. Dr. Henry Imshaug determined the symbiont lichen as *Buellia stillingiana* Steiner and Dr. Stanley Hughes determined the conidial stage as *Sporidesmium folliculatum* (Corda) Mason & Hughes (Can. Jour. Bot. **31**: 609. 1953). I wish to thank Dr. I. Mackenzie Lamb and Dr. H. L. Barnett for stimulating discussions of this project. Cultures of the lichen fungus have been distributed to Centraalbureau voor Schimmelcultures in Baarn, The Netherlands.

by germinating ascospores ejaculated from apothecia of a West Virginia collection on *Acer rubrum* (Hale, No. F-1a, preserved at the Smithsonian Institution). A polyspore inoculum grew extremely rapidly on malt extract-yeast extract agar (10:1:20 gm/l). Within a month the whitish woolly colonies reached a diameter of nearly 1.5 cm and soon turned gray to black, the result of abundant formation of conidia over the whole mycelium.

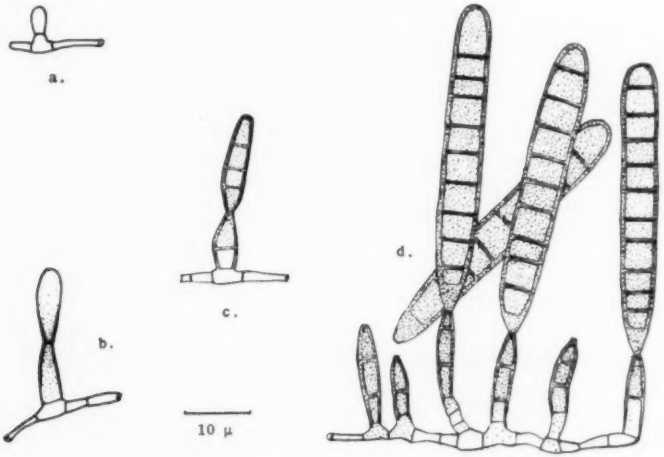

FIG. 1, a–d. Stages in the development of conidia of *Buellia stillingiana*.

The conidia originate from protuberances on vegetative hyphae (FIG. 1, a). After an initial cell divides transversely, the lower half rapidly develops into a brown, 1–3-septate conidiophore (FIG. 1, b, c) which at maturity (FIG. 1, d) attains a length of 10–20 μ and a width of 3–4 μ. The upper half of the initial elongates and divides transversely to form a terminal brown conidium (FIG. 1, b–d). The average dimensions of 100 mature conidia are 6.3 × 56 μ with ranges of 5–7 and 44–79 μ. There are 6–14 transverse septa with an average number of 10. As a rule the conidia are 9 times as long as wide.

When conidia are transferred to the nutrient agar medium, germ tubes emerge from both ends and several intermediate cells within 24 hours at 20–25° C. The tubes branch profusely, and a white woolly colony, identical with that from an ascospore culture, is visible in about 10 days. Sporulation occurs in 10–14 days at 25°.

If these conidia are sought among the Fungi Imperfecti, they appear to be morphologically identical with *Sporidesmium folliculatum* (Corda) Mason and Hughes, a common saprophyte on wood in northeastern North America. This discovery suggests that *S. folliculatum* and perhaps other saprophytic imperfect fungi represent the conidial stages of some fungi which can either parasitize algae, forming symbiont lichens (as *Buellia stillingiana*), or live autonomously as saprophytes. The sexual stage would be achieved in the symbiotic union, the asexual stage in the saprophyte. Of course morphological similarity is not positive proof that the conidial stages of *Sporidesmium folliculatum* and *Buellia stillingiana* belong to the same fungus. Further studies on the physiology, reproduction, and lichenization of each stage will be essential for a final solution.

It will be profitable to search for conidial stages of many more lichen fungi in pure culture and study their relation to recognized imperfect fungi.

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PERITHECIAL INITIALS OF CHAETOMIUM [1]

W. C. WHITESIDE

(WITH 9 FIGURES)

Between 1875 and 1941, observations by at least nine different investigators have been published on perithecial initiation in the genus *Chaetomium*. The early works were concerned largely with the recognition and description of the ascogonium. Van Tieghem (**1875**) described a coiled ascogonium subtended by a ramifying hyphal branch which formed the perithecial wall. In a report the following year, Van Tieghem (**1876**) indicated that he had studied *Chaetomium murorum* Corda, *C. indicum* Corda, and several other species described as new. Zopf (**1881**), in his illustrated monograph, failed to recognize the existence of a definite ascogonium in *Chaetomium globosum* Kunze, although there is evidence to indicate he may have observed such a structure. It should be noted that *Chaetomium globosum* Kunze he renamed *C. Kunzeanum* Zopf, the name that has been used for the species by many subsequent workers, but not recognized as valid in the important monographs of Chivers (**1915**) and of Skolko and Groves (**1953**). In the following discussion the nomenclature as used by the various workers is retained, althought this may not agree with current taxonomic usage. The investigations of Eidam (**1883**) and Oltmanns (**1887**) on *Chaetomium Kunzeanum* Zopf confirmed Van Tieghem's observations. However, Zukal (**1886**) cultured *Chaetomium crispatum* Fuckel, a species having monostichous spore arrangement, on slices of potato and was unable to observe ascogonia. Dangeard (**1907**) studied perithecial development in *Chaetomium spirale* Zopf and found a coiled ascogonium similar to what he had observed in *Chaetomium Kunzeanum* Zopf.

The studies by Vallory (**1911**) and Greis (**1941**) were primarily

[1] This paper is based on a portion of a thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Botany in the Graduate College of the University of Illinois. The writer is indebted to Dr. Leland Shanor, formerly Professor of Botany at the University of Illinois and now Head of the Department of Biological Sciences at the Florida State University, for direction of the research reported in this paper and to Dr. G. W. Martin, Visiting Professor of Botany at the University of Illinois, for suggestions on the preparation of the manuscript.

cytological. Vallory maintained a sexual phenomenon was lacking at the beginning of perithecial formation in *Chaetomium Kunzeanum* Zopf. In contrast, Greis reported functional antheridial structures for *Chaetomium Kunzeanum* Zopf and *C. bostrychodes* Zopf. Page (**1939**) published a study on the life history of certain coprophilous fungi. Several species of *Chaetomium* were included: *C. globosum* Kunze, *C. murorum* Corda, *C. caprinum* Banier, *C. bostrychodes* Zopf, and *C. cochliodes* Palliser. The perithecial initials of these species were described as resembling those of *Sordaria* but smaller in size and with more complicated coilings.

It is apparent from the above review of the literature that the perithecial initials of not more than ten out of the almost 50 species of *Chaetomium* recognized in the latest taxonomic study, that of Skolko and Groves (**1953**), have been studied. Aside from the somewhat questionable observations of Zopf and Zukal, the descriptions and drawings of the ascogonial structures by the various workers are essentially the same, although sometimes with variable interpretation.

The perithecial hairs in the ostiolar region, depending upon the species of *Chaetomium*, assume various degrees of coiling, undulation, flexing, or branching. The ascus is always evanescent and the form is typically clavate with irregular spore arrangement, but in a few species it is cylindrical with spores monostichous. In view of this considerable range of structural characteristics, the possibility of ascogonial variation was considered, especially as it might relate to these more evident morphological characters. Cultures of nine species were available for examination, most of which were supplied originally by Dr. L. M. Ames. Observations were based either on study of poured plate cultures stained with acid fuchsin or on drop cultures, or both. All drawings were made with the aid of a camera lucida.

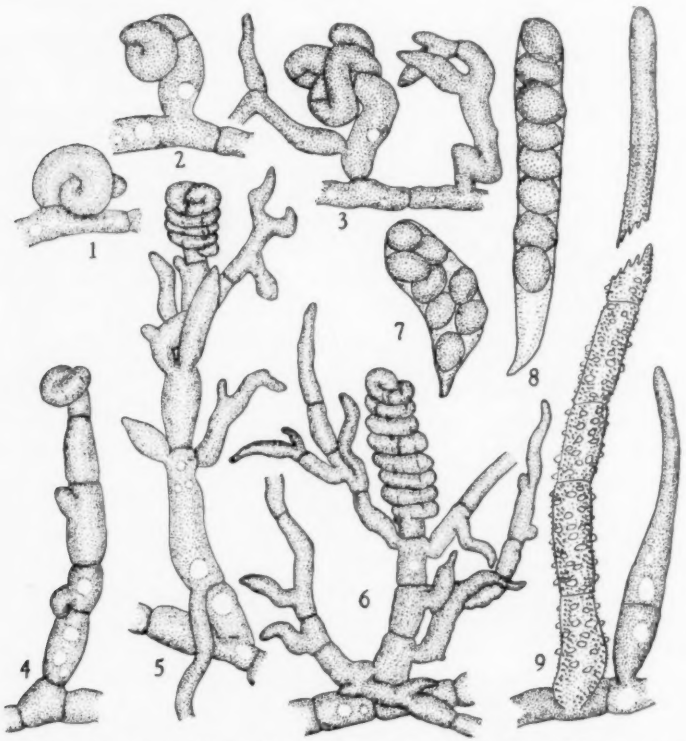
Two patterns of ascogonial development were observed, which suggests that the genus may not be the single phylogenetic unit that is indicated by the use of one generic name. Most of the species studied were found to have perithecial initials similar to those of *Chaetomium globosum* Kunze, but two, typified by the recently named *C. brasiliense* Batista & Pontual, possess a second distinct type which apparently has not been described previously for the genus.

THE CHAETOMIUM GLOBOSUM PATTERN OF ASCOGONIAL DEVELOPMENT

Chaetomium globosum Kunze is selected as representative of what is apparently the more frequent pattern of perithecial initiation in the genus *Chaetomium*. Most of the literature on perithecial development

in the genus is concerned partly or wholly with *Chaetomium globosum*, usually under the synonym *C. Kunzeanum* Zopf, and, therefore, its present study serves as a point of reference to these earlier works.

The first stage of perithecial initiation in *Chaetomium globosum* involves a specialized bent hyphal branch, the ascogonium, of about the same diameter as the hypha from which it originates. The ascogonium may be sessile, or it may occur at the end of one or a few cells (FIGS. 1, 2). The tip elongates and, as it does, coils about itself, the apex



FIGS. 1–9. Ascogonia of *Chaetomium*. 1–3. *Chaetomium globosum*. 1, 2. Early stages of ascogonium formation. 3. A more advanced stage of ascogonial coiling, along with two branched hyphae which will develop into part of the perithecial wall. 4–6. *Chaetomium brasiliense*. 4. A young ascogonium showing the stalked condition characteristic of this species. Two hyphal branches are developing from stalk cells. 5, 6. More advanced developmental stages. 7. *Chaetomium aureum*, ascus. 8, 9. *Chaetomium brasiliense*. 8. Ascus. 9. Mature mycelial hair (left) and a very young mycelial hair (right). All approximately × 1000.

twining tightly about or in between other coils in an irregular course. While the ascogonium is developing, additional specialized hyphal branches, usually several in number, originate from adjacent mycelial cells and from the proximal cells of stalked ascogonia (FIG. 3). These hyphal branches ramify around the coiled ascogonial structure, forming the perithecial walls and subsequently the perithecial hairs.

The ascogonia of *Chaetomium aterrimum* Ellis and Everhart, *C. ochraceum* Tschudy, *C. funicolum* Cooke, *C. dolichotrichum* Ames, as well as those of *C. globosum*, were found to resemble one another so closely that it is impossible to differentiate these species on the basis of this character. Both *Chaetomium pachypodioides* Ames and *C. murorum* Corda appear to have this same developmental pattern, although their perithecial initials are somewhat distinctive in appearance.

No correlation could be observed between this type of ascogonium and any particular form of the terminal perithecial hairs, and an analysis of earlier studies suggests this same conclusion. Specifically, *Chaetomium funicolum* and *C. dolichotrichum* possess essentially straight, dichotomously branched hairs. On the other hand, *Chaetomium globosum, C. ochraceum, C. pachypodioides,* and *C. aternimum* have sinuous to distinctly coiled hairs, and *C. murorum* has flexed hairs with circinate tips. In all these species the ascus is clavate with irregular spore arrangement.

THE CHAETOMIUM BRASILIENSE PATTERN OF ASCOGONIAL DEVELOPMENT

Chaetomium brasiliense Batista & Pontual and *C. aureum* Chivers show the second pattern of ascogonial development. In these two species, perithecial initiation is instituted by a hyphal branch with a bent apex, the branch consisting of one to several somewhat elongate cells (FIG. 4). In *Chaetomium brasiliense* these stalk cells have a greater diameter than the hypha from which they take their origin, but this is not true for *Chaetomium aureum*. The considerable length of the ascogonial stalk contrasts with the sessile to variably stalked condition occurring in the *Chaetomium globosum* pattern. The ascogonial tip elongates and begins to coil about itself, typically spiraling downward around the stalk cells in a symmetrical pattern (FIGS. 5, 6). The maximum number of whorls observed was about eight. Septations in the ascogonium were found to be somewhat difficult to observe, but examination of a great many fairly advanced ascogonia of *Chaetomium brasiliense* revealed the presence of two or three septa each. Specialized hyphal branches emerge from the stalk cells, usually close to the septa, and form the perithecial wall which encloses the coiled ascogonium.

Variations in this pattern were observed to be common. Often the ascogonium would twine about itself in an irregular path that resembles the condition in *Chaetomium globosum*. In *Chaetomium brasiliense*, the ascogonial tip was occasionally seen to coil in the characteristic spiral manner around a neighboring vegetative hypha rather than around the stalk. This condition was not observed in *Chaetomium aureum*, but in this species the apex of the ascogonium sometimes was observed to have grown away from the stalk cells, although no union with other structures was noted.

A distinctive mycelial character occurring in both *Chaetomium brasiliense* and *C. aureum* should be noted. Darkly staining hairs, apparently independent of the perithecia, originate from many of the vegetative hyphae, especially those occurring at the surface of the substrate (FIG. 9). These hairs, which closely resemble the lateral perithecial hairs, are nonseptate when young but soon develop septations. They are especially noticeable in younger cultures before perithecial development has proceeded very far. No such mycelial hairs were noted in *Chaetomium globosum* or in the other observed species having the same pattern of ascogonial development. These mycelial hairs, therefore, possibly might be regarded as a characteristic indicative of an isolate having perithecial initials of the *Chaetomium brasiliense* pattern.

These seems to be no relationship between this pattern of ascogonial development and the terminal perithecial hairs. In *Chaetomium brasiliense* the hairs are coiled, while in *C. aureum* they are slightly to distinctly arched with straight to hooked apices, depending upon the culture medium employed. It is, however, the dissimilarity of the ascus in the two species that is the more noteworthy. In contrast to what might be expected of a character with as much significance attached to it as the ascus, there is apparently no correlation of the ascus form with the common ascogonial pattern that occurs in *Chaetomium brasiliense* and *C. aureum*. *Chaetomium aureum* possesses the typical clavate ascus with irregular spore arrangement (FIG. 7), while *C. brasiliense* has a cylindrical ascus with spores monostichous (FIG. 8).

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OBSERVATIONS ON CHYTRIDIACEOUS PARASITES OF PHANEROGAMS. VI. RESTING SPORE GERMINATION IN PHYSODERMA (UROPHLYCTIS) PLURIANNULATUM [1, 2]

FREDERICK K. SPARROW

(WITH 1 FIGURE)

The resting spores of relatively few of the fifty or more recognized species of *Physoderma* (including for the moment, at least, those assigned to *Urophlyctis*) have been germinated. Any information we can obtain on this process is of interest, particularly with respect to the light it may possibly throw on generic differentiation within the family Physodermataceae. The fungus under consideration is of especial interest since it has been assigned by some investigators to the genus *Urophlyctis,* by others to *Physoderma.* If one is impressed with the morphological similarity of species of *Urophlyctis* to those of *Physoderma* and does not consider the nature of the host reaction significant as a taxonomic character, then *Urophlyctis* is, indeed, as Jones and Drechsler (**1920**) first pointed out, a superfluous taxon. If, however, one takes into account the striking differences in host reaction produced by various members of the group, *Urophlyctis* becomes a convenient repository for all gall-producing species, whereas those forming only pustules, streaks or lesser manifestations on the host may be placed in *Physoderma.*

The fallacy of basing a generic distinction on the reaction of the host tissue has been pointed out by Jones and Drechsler (**1920**), and, since various structural differences between species of the two groups have one by one been found non-existent, merging of the two has been an obvious course.

Since, as has been indicated, resting spore germination is so little known among species of *Physoderma* and *Urophlyctis,* a study of this process in gall- and non-gall-forming species might well show some real differences between them and provide a suitable basis for distinction.

The present writer has already (**1946, 1947**) presented details of the

[1] Acknowledgment is made to the National Science Foundation for its support of these studies.

[2] Paper No. 1059, Department of Botany, University of Michigan.

germination of the resting spores of two non-gall-forming species, namely *Physoderma menyanthis* on the buckbean and *P. maydis* on maize. These also serve to recall that in this group of fungi either the outer resting spore wall is fractured by the gradually protruding "endosporangium," or is dehisced in a clear-cut, circumscissile fashion. The former method is characteristic of *P. menyanthis,* the latter of *P. maydis.* Recent studies on British material of *P. menyanthis* indicate beyond question the absence of any circumscissile dehiscence of a lid.

Physoderma pluriannulatum occurs sporadically in the vicinity of Ann Arbor on *Sanicula marylandica* and *S. gregaria.* It forms on these plants conspicuous crateriform, often lead-colored galls on the above ground parts, primarily the leaves and inflorescences. The general features of the parasite have been described by Jones and Drechsler (**1920**) and by Karling (**1950**). The latter author has also included fragmentary observations on resting spore germination and zoospore production not touched upon by Jones and Drechsler.

In the autumn of 1954 host material containing mature resting spores of *Physoderma pluriannulatum* was buried in a plastic, porous container beneath soil in the woodlot in which the infected *Sanicula* was growing. After overwintering, resting spores were separated from the decayed host tissue and some were placed in distilled water and others in sterilized soil water. Samples were left at 8°, 15° and 22–25° C (room temperature). Best germination was obtained at 8° C and in both soil or distilled water where, after 96 hours, 20–25% (rough estimate) of the resting spores had germinated. At this temperature spores commenced to germinate after 30 hours and continued to do so up to 100 hours after being placed in water. At 15° C, only 5% of the spores were found to have germinated whereas at room temperature no germination occurred.

The first noticeable sign of the process of germination is the gradual disappearance after 20–24 hours in water of the large central vacuole so conspicuous a feature of the mature resting spore (FIG. A). The contents now become somewhat homogeneous. There ensues a gradual swelling of the spore contents which soon become charged with numerous small regularly spaced vacuoles outlined by circles of minute refractive droplets (FIG. B). Coincident with this swelling, irregular fissures appear on the flattened wall of the spore which mark areas of fracturing. The endosporangium now begins to protrude through an irregular opening and continues its extension until its apex is usually beyond the tattered opening in the outer wall (FIGS. B, C). During this process the previously mentioned droplets of refractive material coalesce to form

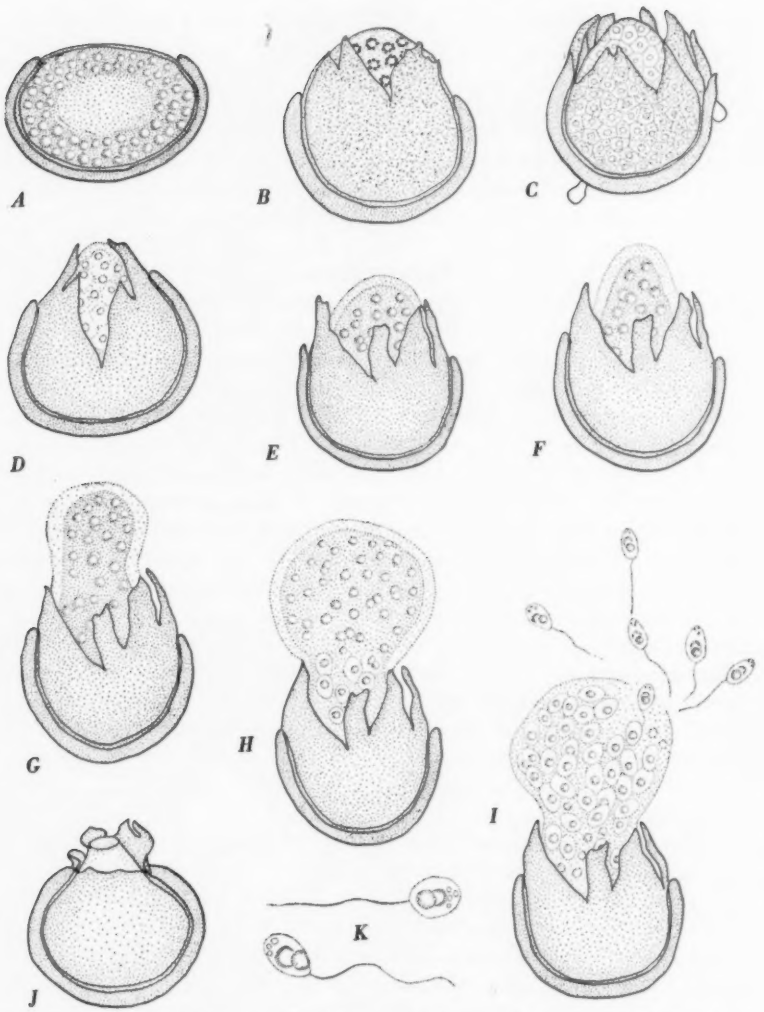

FIG. 1. *Physoderma pluriannulatum.* All figures drawn from living material; all except K × 550. A. Mature resting spore. B. Early stage in germination showing wall of spore fractured by emerging endosporangium, contents of latter showing rings of refractive structures. C. Zoospores cleaved out. D–I. Stages in discharge of endosporangium. Note the zone of gelatinous material which expands as spore mass emerges. In figure I the gelatinous material is ruptured by zoospores as they assume individual motility and swim away. J. Spore

the globules of the zoospores. Lines of spore cleavage may now be visible (FIGS. C, D). This stage in spore germination may take place as early as 30 hours after the spores are first placed in water.

Discharge of the zoospores is preceded by the gelatinization and swelling of the more distal part of the somewhat finger-like extrusion of the endosporangium (FIGS. E–G). The greater part of the spore mass oozes out within this matrix with no individual motion of the zoospores or their outlines visible (FIG. H). When most of the zoospores have left the confines of the endosporangium, individual motility is initiated and the first ones to escape free into the water go through definite fissures in the gelatinous endosporangial material (FIG. I). A general deliquescence of this gelatinous matrix allows the remainder freely to escape. The more proximal unmodified part of the endosporangium remains as a somewhat collapsed structure protruding through the irregular fissure in the dark exospore of the resting spore (FIG. J).

The zoospores are nearly spherical or commonly ellipsoidal with a conspicuous somewhat basal eccentric colorless oil globule, a prominent nuclear cap and a few small refractive anterior globules (FIG. K). They are predominantly $7 \times 5\,\mu$ and bear a posterior, whip-lash type of flagellum $25–27\,\mu$ long, and move in a rapid darting fashion. It is to be expected that these upon germination will produce ephemeral sporangia, but such have not as yet been observed.

From this account it is evident that the type of germination is of the *Physoderma menyanthis* type and that gall-formation and type of resting spore germination (whether by lid dehiscence or fracturing) cannot be used, at least in this species, as associated characters in differentiating *Urophlyctis* from *Physoderma*.

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with discharged endosporangium. K. Two zoospores showing anterior granules, nuclear cap, basal oil globule and posterior flagellum (free hand).

LEAF BLIGHT AND STEM DIEBACK OF COFFEE CAUSED BY AN UNDESCRIBED SPECIES OF ASCOCHYTA

ROBERT B. STEWART [1]

(WITH 1 FIGURE)

The organism described below was first observed in September, 1954, when a blight of undetermined cause was seen in the newly established coffee nursery at the Jimma Agricultural Experiment Station, Jimma, Ethiopia.

Petri in 1931 and 1933 observed a species of *Phyllosticta* and one of *Ascochyta* on *Coffea arabica* L. in Eritrea. The writer believes that these are quite possibly forms of the organism described below. The specific epithet refers to the slow maturing habit of the pycnospores.

Ascochyta tarda sp. nov.

Maculis foliorum rotundatis vel irregularibus, brunneolis, quandoque subconcentricis; pycnidiis amphigenis, globosis vel subglobosis, brunneis, 70–110 μ diam.; conidiis ellipsoideis vel breviter cylindraceis, continuis vel uniseptatis, hyalinis, 2–3 × 9–14 μ. Adsunt perithecia (Mycosphaerellae sp.) brunneo-nigra, epiphylla, globosa aut plana, erumpenti-subsuperficialia, 60–75 × 60–90 μ diam.; ascis fasciculatis, hyalinis, clavulatis vel cylindraceis, 7.5–12 × 25–45 μ; sporidiis inequaliter uniseptatis, 4.5–7.5 × 12–15 μ.

COLLECTIONS: On *Coffea arabica* Jimma, Kaffa Province, Ethiopia, September 15, 1954, Robert B. Stewart. TYPE: Bureau of Plant Industry, Mycological Collections, Beltsville, Maryland, U. S. A. Cotype: Herbarium of the Royal Botanic Gardens, Kew, England.

The organism causes a leaf necrosis and dieback of young branches. The pycnidia are globose to subglobose, mostly epiphyllous, brown, and measure 70–110 μ in diameter. The mature spores are oval to short-cylindrical, 1-septate, straight, and measure 2–3 × 9–14 μ. Such spores usually occur late in the life cycle of the organism, and the immature spores may be predorninantly or entirely aseptate, oval, and measure 2–3 × 4–9 μ.

[1] Present address: Department of Plant Physiology and Pathology, Texas Agricultural Experiment Station, College Station, Texas.

A closely associated species of *Mycosphaerella* (not *M. coffeicola* Cke.) is frequently encountered in natural infections. The perithecia are brownish-black, epiphyllous, globose to frequently flattened, subepidermal but only rarely imbedded in the palisade tissue and measure 60–75 × 60–90 μ. The asci are hyaline, clavate to cylindrical, and measure 7.5–12 × 25–45 μ. The ascospores are inequally 1-septate, sometimes extremely so (almost comma shaped), and measure 4.5–7.5 × 12–15 μ.

Attempts to prove a relationship between the above described species were not conclusive, but there is every indication that they are only forms of the same species.

The symptoms of this disease as expressed on both the branches and the leaves are quite similar to brown blight caused by *Colletotrichum coffeanum* Noack. The leaf lesions are somewhat distinctive in that they are usually irregular (they may also be circular), and frequently begin at the apex or form irregular indeterminate lesions which destroy most or all of the leaf, resulting in leaf abscission. The lesions are tan to brown, and may develop concentric rings. The concentric rings appear to represent former margins of the lesion from which the lesion has expanded to destroy new tissue (FIG. 1). The margin is an indefinite water-soaked region in actively expanding lesions, becoming a definite dark or reddish brown border in inactive lesions. The lesions are distinctly necrotic when observed from either surface, but are usually more pronounced when viewed from the upper leaf surfaces.

On young branches the organism causes a typical dieback symptom with the branches blackening and drying from the tip downward. In severe infections entire plants may be destroyed in this manner. These dieback symptoms are not distinctive and can easily be confused with the same symptom caused by other pathogens or from physiological causes. The organism attacks trees of any age, but aside from varietal differences, the disease appears to be most severe on seedlings and upon young transplants up to 3–4 years old. On all mature plants observed the disease caused little more than an occasional leaf spot with no symptoms of apparent seriousness; however, none of these mature trees were of the susceptible varieties, and perhaps the organisms will also be damaging to the mature trees of a susceptible type.

The disease is most prevalent where plants are growing in excessive shade, and no serious expression of this disease has been observed where shade was light or in unshaded plantings of the susceptible varieties.

Leaves of coffee seedlings were inoculated with a water suspension of *A. tarda* from culture on February 8, 1956. Symptoms were evident

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FIG. 1.

by February 22, and some plants were largely defoliated by March 1. The dieback symptom of the young stems was also evident.

There is apparently considerable difference in varietial resistance to the organism. It is interesting to note that out of 49 coffee varieties of three-year-old plants which were rated from 0–5 to indicate the severity of the disease reaction, only one (S-10 Harrar) of the 15 selections from Ethiopia was rated 3; whereas all others were rated from 0–2 indicating they were highly resistant. Several of the introductions from Central and South America and British East Africa were also resistant, but of the remaining 34 varieties, 12 were rated as 3–5. While there were too few trees of each variety to make the above ratings very reliable, they do seem to indicate a difference in this respect.

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FIG. 1. Leaf blight of coffee caused by *A. tarda*. Note the concentric rings on the lesion on the leaf in the lower right. The puckering is the result of drying and is not a disease symptom.

BENJAMIN MINGE DUGGAR: 1872–1956

G. W. KEITT

(WITH PORTRAIT)

Benjamin Minge Duggar died at New Haven, Connecticut, on September 10, 1956, after a short illness. In his passing the Mycological Society of America lost one of its most productive members and biology and agriculture lost one of their most distinguished scientists.

Benjamin Minge, the fourth of six sons of Dr. Reuben Henry and Margaret Louisa Minge Duggar, was born at Gallion, Alabama, September 1, 1872. His early education was in private schools and with tutors. After two years at the University of Alabama, he transferred to the Mississippi Agricultural and Mechanical College, where he received the B.S. degree with first honors in 1891. He received the M.S. degree from the Alabama Polytechnic Institute in 1892 and was Assistant Director of the Canebrake Agricultural Experiment Station at Uniontown, Alabama, in 1892–93. He was granted the B.A. degree in 1894 and the M.A. in 1895 from Harvard University, working under the direction of W. G. Farlow. In 1898, he received the Ph.D. from Cornell University, where his studies were supervised by G. F. Atkinson. He spent two periods of post doctoral study in Europe in 1899–1900 and 1905–06. He was Assistant Botanist, Illinois State Laboratory of Natural History, 1895–96, Assistant Cryptogamic Botanist, Agricultural Experiment Station, and Instructor in Plant Physiology, 1896–00, Assistant Professor, 1900–01, Cornell University; Physiologist, Bureau of Plant Industry, U. S. Department of Agriculture, 1901–02; Professor of Botany, University of Missouri, 1902–07; Professor of Plant Physiology, Cornell University, 1907–12; Research Professor of Plant Physiology, Missouri Botanical Garden and Washington University, 1912–27; Acting Professor of Biological Chemistry, Washington University Medical School, 1917–19; Professor of Plant Physiology and Economic Botany, University of Wisconsin, 1927–43, also part-time Professor of Plant Pathology, 1929–43, Emeritus since June, 1943; Consultant in Mycological Research and Production, Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York, since 1944.

In 1901, he married Marie L. Robertson, who died in 1922. Their children were Marie Louise, Benjamin Minge, Anna St. Julian Guerard,

George Struan-Robertson, and Emily Westwood. In 1927, he married Elsie Rist. They had one daughter, Gene Lorraine.

Possessed of a brilliant mind, untiring interest and energy, singleness of purpose, and unusually broad education and contacts with leading

BENJAMIN MINGE DUGGAR

American and European scientists in the formative period of American science and scientific institutions, Duggar in an exceptional degree compassed the wide fields of biology and agriculture. This brief sketch must be concerned chiefly with the mycological aspects of his work; more extended treatment of his other contributions will be available elsewhere.

Though he would not have considered himself primarily a mycologist, mycology in its broadest aspects was central in his many-faceted contributions throughout his career. His early appointments were concerned chiefly with mycology and plant pathology. He was the author of many papers on plant diseases and of the first textbook on plant pathology published in the United States (Fungous Diseases of Plants, Ginn & Co., Boston, 1909. 508 p.). Among the diseases he studied were some fungus diseases of pear, three fungus diseases of sugar beet, peach leaf curl, diseases incited by *Rhizoctonia* species, and the Texas root rot disease, incited by *Phymatotrichum omnivorum*. His researches on the *Rhizoctonia* and *Phymatotrichum* diseases were especially significant in advancing the recognition and understanding of these very important maladies. He published a comprehensive monograph on *Rhizoctonia* (*Rhizoctonia crocorum* (Pers.) DC. and *R. solani* Kühn (*Corticium vagum* B. & C.), with notes on other species. Ann. Missouri Botan. Garden **2**: 403–458. 1915) and discovered the conidial stage of the Texas root rot fungus, which he transferred from the genus *Ozonium* to *Phymatotrichum* (The Texas root rot fungus and its conidial stage. Ann. Missouri Botan. Garden **3**: 11–23. 1916). His researches on mushroom culture (Mushroom Growing, Orange Judd Co., New York, 1915. 250 p.) largely laid the foundation for an important industry. As Duggar's work progressed, he became increasingly interested in physiology (Plant physiology—present problems. Science **21**: 937–953. 1905; Physiological plant pathology. Phytopathology **1**: 71–78. 1911). When he chose to make plant physiology his chief specialty (Plant Physiology, Macmillan, New York, 1911. 516 p.), a major share of his effort was devoted to the physiology of fungi. He recognized the exceptional suitability of fungi for fundamental physiological investigations and the importance of basic physiological studies for the elucidation of practical problems in agriculture, industry, and medicine. In his work at the Missouri Botanical Garden and Washington University, he and his students published a notable series of papers on the physiology of fungi, and this specialty was cultivated throughout the years he spent at the University of Wisconsin. During his 17 years at Wisconsin, he not only led in an outstanding development in plant physiology, but contributed greatly through his teaching, research, and counsel to progress in the plant sciences and broadly to the advancement of science and education within the University and beyond state and national borders.

A brilliant academic career as teacher, investigator, and counselor was followed by equally brilliant contributions to industrial research and development. In the emeritus years with the Lederle Laboratories, he

brought his mycological and physiological knowledge and talents to bear on the search for new antibiotics. This led to the discovery and development of aureomycin (Aureomycin: a product of the continuing search for new antibiotics. Ann. N. Y. Acad. Sci. **51**: 177–181. 1948; Aureomycin and preparation of same. U. S. Patent No. 2,482,055. 1949) and to a research program that engaged his interest and effort throughout the remainder of his life.

The breadth of Duggar's activities and the quality of his contributions are indicated by the many scientific organizations to which he belonged and the high honors he received. He was a Fellow of the American Association for the Advancement of Science (V.P. Section G, 1925); member of the National Academy of Sciences, American Philosophical Society, Philadelphia Academy of Science, Botanical Society of America (Pres. 1923), American Society of Plant Physiologists (Pres. 1946–47), American Society of Agronomy (V.P., 1908), American Phytopathological Society, American Chemical Society, American Society of Naturalists (V.P., 1928), Society for Experimental Biology and Medicine, Society for Industrial Microbiology (Pres., 1952), American Public Health Association, Torrey Botanical Club, National Research Council (Chairman, Division of Biology and Agriculture, 1925–26), Phi Beta Kappa, Sigma Xi, Phi Sigma; Chairman of Organizing Committee and General Secretary, International Congress of Plant Sciences, Ithaca, New York, 1926; Editor for Physiology, Botanical Abstracts, 1917–26, and Biological Abstracts, 1926–33; Editor, Biological effects of radiation. 2 v. McGraw-Hill, New York, 1936. 2019 p.; Trustee, Woods Hole Marine Biological Laboratory, Woods Hole Oceanographic Institution, and Bermuda Biological Station. He was an honorary fellow of the International College of Surgeons, an honorary member of La Societa Italiana di Ematologia, and a corresponding member of La Societa Lancisiana di Roma. He was awarded the honorary LL.D. degree by the University of Missouri in 1944, the Sc.D. by Washington University in 1953 and by the University of Wisconsin in 1956, the Medal of Honor of Public Education of Venezuela and a medal from The Pasteur Institute in 1951, and in the same year a decoration from Pope Pius XII for his services to mankind.

He was the author or coauthor of three books and many research papers. Especially outstanding among his researches were his contributions to the physiology of fungi; to the nature and properties of viruses; to biological effects of radiation, including quantum efficiency in photosynthesis; and to the discovery and development of aureomycin. His publications were too numerous to be listed here. It is planned that

they will be cited in a memorial that will be published by the National Academy of Sciences.

In his busy life, he found time for activities beyond his science. He was devoted to his family and friends and found relaxation and refreshment in hobbies. He especially enjoyed outings, in which camping, fishing, and botanizing were often combined. He took a keen interest in sports and enjoyed bowling and golfing until his activities were curtailed by his last illness. Another favorite recreation was gardening. Even in his later years he maintained a vegetable garden in which he could work with growing plants and from which he could supply his friends and his own table.

Benjamin Minge Duggar will long be remembered for his distinguished contributions to science and education, and the strong influence that he exercised for more than half a century in biology and agriculture will be sorely missed.

NOTES AND BRIEF ARTICLES

A NEW SPECIES OF LICEA FROM PANAMA

Licea tuberculata sp. nov.

Sporangia sessilia, pulvinata vel subglobosa, 0.2–0.7 mm. diam.; peridiis nigris, tuberculatis, irregulariter dehiscentibus; sporis globosis vel ellipsoideis, levis, paries crassis, pallide flavo-brunneis, 9–11 μ.

Sporangia sessile, pulvinate to subglobose on a constricted base, 0.2–0.7 mm in diameter; peridium black, tuberculate; dehiscence irregular; spores globose or slightly elliptical, smooth, thick-walled, pale yellowish brown under lens, paler at one side, 9–11 μ.

PANAMA CANAL ZONE: Barro Colorado Island, G.W.M. *8548*, TYPE.

This species appeared on excess bark under a *Hypocrea* collected on Barro Colorado Island, August 20, 1952. The bark was put in a sterile petri dish and moistened with sterile carbon water on October 27, 1953. Various fungi and myxomycetes appeared on it, but none worthy of particular attention developed and the culture was allowed to dry in the spring of 1954. In October, 1954, it was remoistened and *Perichaena depressa* Libert appeared at intervals for a period of over three months. The *Licea* was not recognized until January, 1955, although it had been seen earlier and dismissed as a small, pulvinate pyrenomycetous stroma, which it strongly suggests. It first appears as a creamy white plasmodial mass about 0.5 mm in diameter on the surface of the bark. This enlarges somewhat, up to a maximum of about 1 mm, and dark spots—the future tubercles—appear on the creamy surface. Maturation proceeds slowly, the cushion becoming orange-brown, then maroon and finally black, contracting in the process. The black tuberculate bodies are difficult to distinguish on the surface of the bark, but when bits of the bark were transferred to other dishes on moist filter paper, the plasmodium appeared to migrate, although no migrating plasmodium was seen. At any rate, new plasmodial masses appeared on the filter paper, often 1 cm or more from the bark, and developed into typical sporangia. Some small fruitings were gathered as late as July, 1955.

The color of the spores is in the outer wall, which is often sharply marked off from the colorless, hyaline part of the wall. The protoplast

is usually withdrawn from the inner wall and contains 1–3 prominent vacuoles. A few microtome sections show an umbilicate lower surface, so that the pulvinate appearance is really that of a disk which has been raised in the center.—G. W. MARTIN, Department of Botany, University of Illinois, Urbana.

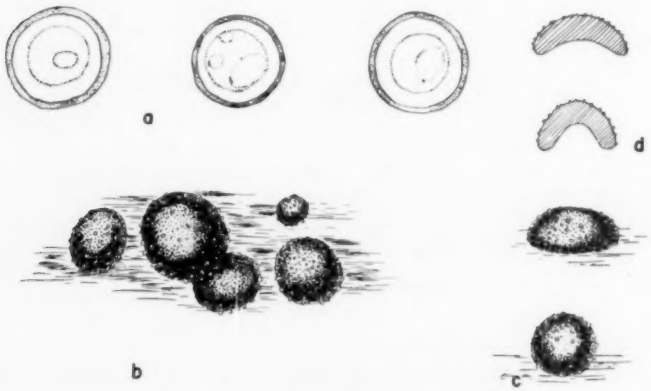


FIG. 1. *Licea tuberculata*. a. Three spores, × 1500. b. Five sporangia, as seen from above. c. Two sporangia, from side. d. Two sporangia in outline, in section, showing basal umbilicus. b–c. × 30.

A METHOD FOR INDUCING GLYCOGEN FORMATION IN THE CELLS OF SACCHAROMYCES CEREVISIAE

When yeast cells are suspended in a sugar solution of low concentration, glycogen granules are deposited in the cytoplasm. From these initially scattered deposits, larger and more dense formations may arise. Lindegren [1] has made similar observations.

The technique described here permits more than the usual accumulation of glycogen reserves within the yeast cell.

A medium containing 5% dextrose and 10% ethanol is placed in a loosely covered glass cylinder. This is inoculated with about $\frac{1}{10}$% of a suspension of bakers yeast (a strain of *Saccharomyces cerevisiae*) and

[1] Lindegren, Carl C. 1949. The Yeast Cell, Its Genetics and Cytology. Chapter 7. Educational Publishers, St. Louis, Mo.

allowed to ferment at room temperature. Although cells may not all react uniformly, occasional inversion of the cylinder will favor development of a more homogeneous population. Progress made by the culture can be followed with the iodine stain described by Townsend and Lindegren.[2] Photomicrographs of these iodine-stained yeast cells present varied patterns of glycogen distribution.

FIGS. 1, 2, 3 show earlier stages of glycogen deposition where the formations are enlarging at expense of the vacuoles. Such accumulations may advance from one end of a cell, crowding the vacuole to the other end (FIGS. 1, 2), or they may expand from a more centrally located

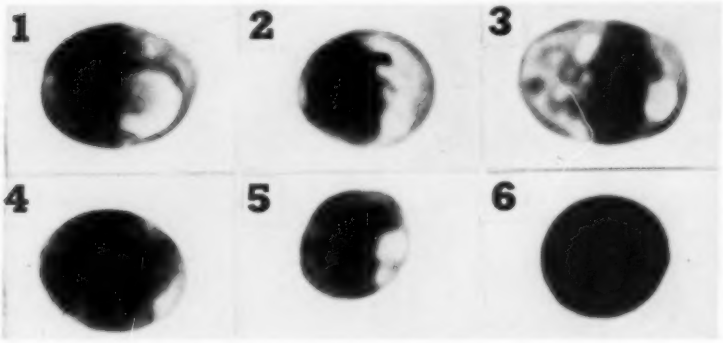


FIG. 1–6. Cells of *Saccharomyces cerevisiae* containing deposits of glycogen. 1, 2, 3. Cells partly filled with glycogen. Vacuoles reduced in size. 4, 5. Cells well-filled with glycogen. Later stages of deposition. 6. Cell in final stage of deposition. Appears entirely filled with glycogen.

position, pushing vacuole lobes toward opposite ends (FIG. 3). The cells in FIGS. 4, 5 have become very nearly filled with glycogen while the cell in FIG. 6 appears completely filled. In this latter instance, the central vacuole has either disappeared or been reduced to submicroscopic size, for it is no longer visible at any focal level.

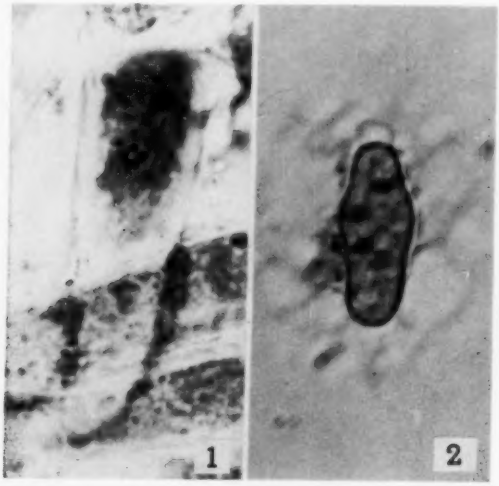
Use of this dextrose ethanol medium has been found to produce extensive glycogen deposition in yeast cells.—G. FRED TOWNSEND, Wahl-Henius Institute, Chicago 14, Illinois.

[2] Townsend, G. Fred and C. C. Lindegren. 1953. Structures in the yeast cell revealed in wet mounts. Cytologia **18**: 183–201.

NOTES ON PHYSALOSPORA ILICIS

Physalospora ilicis (Schl. ex Fr.) Sacc. has long been known as the cause of a leaf spot of *Ilex opaca* Ait. in North America and of *I. aquifolium* L. in Europe. It has been reported [1] by the writer as producing a similar spot on leaves of *I. crenata rotundifolia* Host.

While Ellis made the notation "Pycnidia of Physalospora ilicis" on his exsiccatus of *Phyllosticta ilicicola* (Cke. & Ell.) Ell. & Ev. and Seaver [2] has the statement "Said to be the pycnidial stage of *Physalospora*



FIGS. 1, 2. *Physalospora ilicis.* 1. Young ascus with dividing nucleus in the metaphase I stage, exhibiting four bivalent chromosomes. 2. Ascospore with three nuclei in focus and one nucleus below the plane of focus.

ilicis" in his description of *Phyllosticta ilicicola,* neither these nor more recent workers have produced the one stage from the other, definitely establishing the relationship.

An opportunity for further study of the life cycle of this fungus was made possible when it was isolated in pure culture from infected holly leaves [1] and was found to produce fertile perithecia typical of those described for *Physalospora ilicis.* Monosporic cultures derived from

[1] Driver, C. H. 1952. *Physalospora ilicis* on *rotundifolia* holly in Georgia. Plant Disease Reporter U.S.D.A. **36**: 355.

[2] Seaver, F. A. 1922. Phyllostictales. North Amer. Flora **6**: 24.

25 randomly selected ascospores produced fertile perithecia when grown on corn meal agar and produced fertile pycnidia typical of those described for *Phyllosticta ilicicola* when grown on carrot agar for one to two weeks at room temperature. Reciprocally, monosporic conidial cultures were found to produce fertile perithecia typical of *Physalospora ilicis* when grown on corn meal agar at room temperature for two weeks.

On finding that monosporic cultures would produce the perfect stage, the cytology of the sexual stage of this fungus was investigated. By employing the propiono-carmine smear technique the following observations were made: (1) Groups of asci were produced by crozier formation at the tips of ascogenous hyphae. (2) The terminal ends of mature asci were thin-walled and did not exhibit a pore for spore dispersion. (3) The haploid chromosome number at metaphase of meiosis I appeared to be four (FIG. 1). The morphology of a genome appeared to be one large chromosome, two intermediate chromosomes and a single very small chromosome. (4) Ascospores were uninucleate at the time of their origin. (5) Mature ascospores were multinucleate, having from two to four nuclei before being expelled from the ascus (FIG. 2).

The results of this investigation demonstrate the following points: (1) *Physalospora ilicis* is the perfect stage of *Phyllosticta ilicicola*. (2) This fungus is truly homothallic. (3) The complete life cycle of this member of the genus *Physalospora* can be reproduced in pure culture on standard laboratory media. (4) The chromosome number and morphology of this species is similar to that reported by Wheeler and Driver [3] for *Glomerella cingulata* (Stonem.) Spauld. & v. Schrenk, which could indicate a natural relationship between the two represented genera in one respect. However, the occurrence of the thin-walled mature ascus tip without a pore and the multinucleate condition of the mature ascospore exhibited by this species and *Physalospora obtusa* (Schw.) Cooke [4] are thought to represent natural differentiating generic characteristics between *Physalospora* and *Glomerella*.

Specimens of the conidial stage of this fungus on *rotundifolia* holly leaves have been filed in the following herbaria, C.H.D. No. 869, Department of Plant Pathology, University of Georgia; C.H.D. No. 873, Department of Plant Pathology, Cornell University; and C.H.D. No. 873, in the National Fungus Collections of the USDA. Pure cultures of *Physalospora ilicis* (Schl. ex Fr.) Sacc. have been placed in The

[3] Wheeler, H. E. and C. H. Driver. 1953. Genetics and cytology of a mutant, dwarf-spored *Glomerella*. Amer. Jour. Bot. **40**: 194–207.

[4] Aycock, R. 1951. Some phenomena in *Physalospora obtusa*. Phytopath. **41**: 459–465.

American Type Culture Collection, Washington, D. C., and the Centraalbureau voor Schimmelcultures, Baarn, Holland.

The writer gratefully acknowledges the constructive criticism of this note by Dr. J. H. Miller.—CHAS. H. DRIVER, Research Department, International Paper Company, Mobile, Alabama.

NEW ABSTRACT JOURNAL

North Carolina State College recently announced the establishment of the world's first Tobacco Literature Service and the publication of its first issue of TOBACCO ABSTRACTS. The Service, which is a joint undertaking of the D. H. Hill Library and the Agricultural Experiment Station, will assemble and publish citations and abstracts of the world literature on cultivated tobacco and the genus Nicotiana. It also will provide library staff service at State College for tobacco research workers. The Literature Service developed as an outgrowth of a need expressed by tobacco scientists for assistance in assembling and reviewing technical literature for tobacco in the various fields of science, including mycology. The monthly issues of TOBACCO ABSTRACTS are being made available to libraries in the United States and certain other countries. Subscription rates are $7.00 per year in United States and $10.00, foreign. Address TOBACCO ABSTRACTS, D. H. Hill Library, N. C. State College, Raleigh.

CHANGE OF ADDRESS

As of June 15, the address of the Editor-in-Chief will be Department of Botany, State University of Iowa, Iowa City, Iowa.

REVIEWS

A MANUAL OF SOIL FUNGI, by Joseph C. Gilman. Revised, second edition. x + 450 pp. 152 figs., 13 plates. Iowa State College Press, Ames. 1957. Price, $7.50.

Since its publication in 1945, Gilman's book on soil fungi has been one of the most frequently consulted volumes in mycological laboratories the world over. As it includes reference to a large number of common saprobic fungi, not by any means restricted to the soil, it has been used freely by students who come across these things in the course of culture work of many different kinds. It is therefore highly gratifying that it should have been revised in the light of the new information which has accumulated in the twelve years since it first appeared.

The new edition has been enlarged to 450 pages from the 392 of the earlier volume, with the inclusion of 32 additional genera and 142 additional species as well as the exclusion, for various reasons, of some species recognized in the first edition. Line drawings have been added for most of the newly admitted genera, but the old cuts used in the first edition, many of them not too satisfactory, have been retained. In addition, a frontispiece and 13 plates, with 85 reproductions from photomicrographs, have been added. Most of these are helpful; in some cases, those of the Aspergilli and Penicillia on Plates VIII to X for example, they are not likely to supplement the better figures in available manuals.

The paper is of better quality than in the former edition and the binding appears to be stronger. These are important considerations in a book which is likely to receive constant use.

As the author indicates in his introduction, the problem of what fungi are to be regarded as regular components of soil complexes is far from having been solved. Perhaps it never will be. But in view of the steadily increasing interest in fungi as related to soils and in the same fungi as they occur elsewhere, there can be no question of the wide usefulness of the new volume.—G. W. MARTIN.

TOBACCO DISEASES, by J. C. F. Hopkins. xi + 178 pp., colored pl. + 91 figs. Commonwealth Mycological Institute, Kew, Surrey. 1956. Price, 35 shillings (about $4.90).

This well illustrated handbook is designed to serve plant pathologists, extension officers and planters in the tobacco growing areas of Africa. The author draws on his experiences of more than 30 years to

bring up to date an account of tobacco diseases in Africa and methods of dealing with them. The author's contention that the control of plant diseases through the breeding and growing of resistant varieties has been over-emphasized in recent years will be disputed by many plant pathologists, plant breeders and growers, especially in those areas where disease-resistant varieties have played major roles in the tobacco industry. However, the emphasis he places on other control measures such as hygiene, quarantine, seed treatment, protection, eradication and destruction is well justified and he treats these subjects in an exhaustive fashion. The author devotes 45 pages to care of seedbeds and plant hygiene, thereby recognizing and emphasizing the importance of production of healthy, vigorous seedlings and sanitary measures in the field.

Major diseases such as brown spot (caused by *Alternaria longipes*), frogeye (caused by *Cercospora nicotianae*), mosaic (TMV) and wildfire (caused by *Pseudomonas tabaci*) are treated in detail, with proper emphasis on control measures. Other diseases occurring in Africa are in general adequately covered.

Since this is a handbook for grower use it is regrettable the author does not treat nematode diseases, which are known to be serious in Africa, nor does he discuss the effects of cropping sequences on disease development and control. He does not discuss some of the more recent developments in control of diseases in the plant bed such as the use of methyl bromide and other chemical soil treatments. Although a bibliography of more than 150 titles is included there are important omissions, including the work of the Japanese on anthracnose, the Canadians on root rots, the English on viruses and such important work in the United States as: 1) Clayton and co-workers on breeding for resistance to Granville wilt and black shank, 2) Anderson and others on barn rot, and 3) Steinberg on frenching.

In every book a few mistakes are inevitable. On page 62 the correct name for the wildfire organism is *Pseudomonas tabaci* not *tabacum*, and on page 135 the virus causing ring spot, which the author states is undetermined, has been determined, isolated and studied by electron microscopy.—D. E. ELLIS, Plant Pathology, North Carolina State College.

A GLOSSARY OF MYCOLOGY, by Walter H. Snell and Esther A. Dick. xxxi + 171 pp., 15 pl. Harvard University Press, Cambridge, Mass. 1957. Price, $5.00.

Snell's "Three thousand mycological terms," published in 1936, has long been unavailable and most of the extant copies are badly worn from

constant use. The present greatly expanded volume is, therefore, most welcome and will undoubtedly be even more useful than its predecessor. The number of definitions has been increased to nearly 7000 and the illustrations, by H. A. C. Jackson, who drew them for the earlier volume, have been increased from 12 to 15 plates, with a corresponding increase of the total number of diagrams. The book is produced by an offset process in double column format, the print is clear, the paper tough and the binding strong—all important features in a constantly used reference book.

As the author notes in his preface, many terms used in mycology are not strictly mycological but it has seemed well to include them, in some cases with reference to their special mycological applications. A fairly extensive spot check has failed to reveal any terms missing which should have been included, or any definitions to which serious objection might be made. Only extensive use can reveal the existence of such, but the care with which the work has been compiled makes it seem unlikely that many will be found.

To the very pertinent quotation from Dr. Johnson in the earlier volume has been added another from the same distinguished source: "Every other author may aspire to praise; the lexicographer can only hope to escape reproach." So far as the present reviewer is concerned, the authors have escaped reproach.—G. W. MARTIN.

FUNGI OF WEST PAKISTAN, by Sultan Ahmad. Biological Society of Pakistan, Monograph No. 1. Government College, Lahore, Pakistan. 126 pp. + map. 1956. Price, Rs. 10 (about $2.00).

The reports on Indian fungi by Butler and Bisby and by Mundkur, both published before the establishment of Pakistan, include fewer than 200 species from the area of 300,000 miles represented by West Pakistan. In the present work the author has increased the number to over 1200 species, but recognizes that this is only a beginning, since extensive regions are still mycologically unknown.

The annotated list of species, arranged in standard taxonomic sequence, is followed by a bibliography, index to genera, host index and map. The volume will be of service in all mycological collections.—G. W. MARTIN.

MANUSCRIPT

Publication in MYCOLOGIA is ordinarily restricted to those who have been members in good standing of the Mycological Society of America for over a year immediately preceding submission of manuscript. Exceptions to this regulation require a favorable vote by a majority of the Editorial Board. When a paper has two or more authors, the person submitting the paper is expected to be a member.

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